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(21) International Application Number: PCT/US97/05701 (22) International Filing Date: 8 April 1997 (08.04.97) (30) Priority Data: 60/014,970 8 April 1996 (08.04.96) US (60) Parent Application or Grant (63) Related by Continuation US 60/014,970 (CIP) Filed on 8 April 1996 (08.04.96) (71) Applicant (for all designated States except US): GLAXO GROUP LTD. [GB/GB]; Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): GEYSEN, Hendrik, Mario [AU/US]; Glaxo Wellcom Inc., Five Moore Drive, Research Triangle Park, NC 27709 (US). KINDER, Daniel, Start [US/US]; Glaxo Wellcome Inc., Five Moore Drive, P.O. Box 13398, Research Triangle Park, NC 27709 (US). WAGNER, Craig, Daniel [US/US]; Glaxo Wellcome Inc., Five Moore Drive, P.O. Box 13398, Research Triangle Park, NC 27709 (US).		(74) Agents: LEVY, David, J. et al.; Glaxo Wellcome Inc., Five Moore Drive, P.O. Box 13398, Research Triangle Park, NC 27709-9602 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: MASS-BASED ENCODING AND QUALITATIVE ANALYSIS OF COMBINATORIAL LIBRARIES		
(57) Abstract <p>The insertion of isotopically labeled portions into solid state combinatorial synthesis constructs followed by mass spectrometer, mass-based nuclear magnetic resonance spectrometry or mass-based infrared spectrometry analysis allows for the physical, non-chemical encoding of large numbers of combinatorial synthesis products.</p>		

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TITLE OF THE INVENTION

MASS-BASED ENCODING AND QUALITATIVE ANALYSIS
OF COMBINATORIAL LIBRARIES.

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BACKGROUND OF THE INVENTION

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15 The present invention generally relates to the field of combinatorial chemistry and in particular relates to encoding the library products of combinatorial synthesis.

20 Recent trends in the area of research for novel chemical and especially pharmacological agents have been concentrated on the preparation of so-called "chemical libraries" as potential sources of new leads for drug discovery. Chemical libraries are intentionally created collections of differing molecules which can be prepared either synthetically or biosynthetically and screened
25 for biological activity in a variety of different formats. One can have libraries of soluble molecules; libraries of compounds tethered to resin beads, silica chips or other solid supports; or recombinant peptide libraries displayed on bacteriophage or other biological display vectors. Chemical libraries are advantageously
30 made by using techniques from the field of combinatorial chemistry. The field of combinatorial chemistry is a synthetic strategy which leads to large chemical libraries. Combinatorial chemistry can be defined as the systematic and repetitive covalent connection of a set of different building blocks of varying

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structures to each other to yield a large array of diverse molecular entities.

- Traditionally, new medicinal chemical lead structures have
5 originated from the isolation of natural products from
microbiological fermentations, plant extracts, and animal sources;
from screening of pharmaceutical company compound databases;
and more recently through the application of both mechanism-
10 based and structure-based approaches to rational drug design. All
of these methods are relatively expensive. Recent cost studies
suggest that the average cost of creating a new molecular entity in
a major pharmaceutical company is around \$7,500 per
compound, using the traditional chemical synthesis technology
15 that requires more or less constant hands-on manipulation of
reagents and apparatus and the attention of a chemist.
Furthermore, the advent of high throughput automated techniques
has made possible the robotized screening of in excess of
hundreds of thousands of individual compounds per year, per
20 drug target. The availability of this capability, combined with the
relatively high cost of more traditional hand crafted chemistry has
caused a global shift in emphasis toward the concept of mass
production, which is an industrial concept that can be put into
being using the approach of combinatorial chemistry.
- 25 The inefficiencies of hand crafted chemistry are thus largely
addressed by a switch to the concept of using combinatorial
chemical technologies for rapidly synthesizing compound
collections. Thus, by employing a building block approach and by
systematically assembling these blocks in many combinations
30 using chemical procedures it is possible to create chemical
libraries as vast populations of molecules. An essential starting
point for the generation of molecular diversity is an assortment of
small, reactive molecules which may be considered chemical
building blocks. The universe of structural diversity accessible

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through assembly of even a small set of building-block elements is potentially very large, and unleashing the power inherent in the building block approach is crucial to the success of the combinatorial method. The building block argument is easily
5 illustrated as follows. Theoretically, the number of possible different individual compounds, N prepared by an ideal combinatorial synthesis is determined by two factors; the number of blocks available for each step " B " and the number of synthetic steps in the reaction scheme, s . If an equal number of building
10 blocks are used in each reaction step, then $N = B^s$. If the number of blocks that one desires for each step varies (e.g. b , c , d in a three-step synthesis), then $N = bcd$.

From the above, it can be seen that a relatively conservative
15 combinatorial synthesis procedure involving 20 blocks in a three step synthesis process will produce $20^3 = 8000$ compounds. This relatively generous production output then raises the next question, which is, how will the compounds be identified? For example, a typical combinatorial synthesis technique is that of the
20 split synthesis. As an example of split synthesis in the solid state synthesis of peptides, a batch of resin support (typically small resin beads) is divided into n fractions, coupling a single monomer amino acid to each aliquot in a separate reaction, and then thoroughly mixing all the resin particles together. Repeating
25 this protocol for a total of x cycles can produce a stochastic collection of up to n^x different molecules, as governed by a hypergeometric distribution. To ensure representation of the majority of possible ligands one needs to begin with a multiplicity of beads. A typical value would be ten times as many beads as the
30 desired number of ligands. Theoretically a set of every possible combination of the building blocks exists in the aliquots. In order to determine the composition of a particular compound which is found to be of interest, one could proceed with direct ligand structural analysis, preferably on a mass spectrometer, on a

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species-by-species basis. A typical combinatorial synthesis now typically takes place on a reaction plate having from 96 to 2,304 reaction wells. One identifies the product compounds of true interest by a positive response in an appropriate assay. However, even after assays are run that will greatly reduce the number of compounds as having been non-active in the assay, the problem with a conventional mass spectrometer analytical approach is that many individual analysis trials are required, there may only be very small quantities of material available after running a combinatorial synthesis, and overall turn around time may be quite lengthy. A need exists, then to somehow label compounds as they are going through their combinatorial steps. Where compounds are, for example, tethered to resin beads, prior art solutions to the problem have included attaching chemical identifier tags to the beads coincident with each block coupling step in the synthesis. The different chemical properties of each tag would then convey which building block was coupled in a particular step of the synthesis, and the overall structure of a ligand on any bead could be deduced by "reading" the set of tags on that bead, in effect having encoded the bead.

Tags should ideally have a highly discrete information content, be amenable to very high sensitivity detection and decoding, and must be stable to reagents used in the ligand synthesis. Prior art tags attached onto beads have included nucleotides, peptides, or a combined series of hydrocarbon homologs and polychlorinated aromatics. Single stranded oligonucleotides are built on resin beads upon which peptide synthesis is being performed and which are subsequently amplified through polymerase chain reaction and sequenced. Another technique is one where orthogonally differentiated diamine linkers are used in the construction of soluble chimeric peptides comprising a "binding " strand and a "coding" strand. As amino acid monomer building blocks are coupled to the binding strand, this is recorded by building an

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amino acid code onto the "coding " strand. The sequence of the coding strand is then resolved by Edman degradation. One problem with this approach is that it requires an extra chemical step for every step taken in the construction of the library.

- 5 Another problem with this approach is that it requires requires orthogonal synthetic procedures for building up a tag in conjunction with synthesis of ligands, i.e., it requires the addition of like moieties, whereas there is very great interest in discovering methods for producing compounds which are not
- 10 limited to sequential addition of like moieties. Such methods would find application, for example in the modification of steroids, antibiotics, sugars, coenzymes, enzyme inhibitors, ligands, and the like, which frequently involve a multi-stage synthesis in which one would wish to vary the reagents and/or
- 15 reactions conditions to provide a variety of compounds. In such methods the reagents may be organic or inorganic reagents, where functionalities may be introduced or modified, side groups attached or removed, rings opened or closed, stereochemistry changed, and the like. For such a method to be viable, however,
- 20 there needs to be a convenient way to identify the structures of the large number of compounds which result from a wide variety of different modifications.

- 25 A technique that is useful for the screening of nonsequenceable organic molecules prepared by multistep combinatorial synthesis uses a series of gas chromatographically resolvable halocarbon derivatives as molecular tags which, when appended to reactive groups on the bead surface, can constitute a binary code that reflects the chemical history of any member of a library. Instead
- 30 of the oligonucleotide or peptide coding approaches where the order of assembly of the chemical building blocks for any library member is preserved in the sequence of a single cognate tagging molecule, the binary strategy uses a uniquely defined mixture of tags to represent each building block at each particular step of the

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synthesis. Thus a set of N tags can be used to encode the combinatorial synthesis of a library of 2^N different members. After assembly, the tags are photolysed and analyzed by electron capture capillary gas chromatography.

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In all cases, the use of reporter tags complicates synthetic strategies, increases the risk of side reactions and by-products, and yields only indirect evidence of structure. Thus, there is a need to find a way whereby a compound's reaction history may be recorded, and the structure of the resulting compound identified.

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The use of ^{13}C site-specific labels on the ligand itself has also been used, in connection with ^{13}C NMR spectroscopy as a method of monitoring progress in solid state combinatorial synthesis.

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Yet another method is that of using a chip which allows for separate analysis at physically separate sites on the surface of the chip. By knowing what reactant is added sequentially at each such site, one can record the sequence of events and thus the series of reactions. If one then subjects the chip to a screening method for a particular desired characteristic and detects the characteristic one can readily determine the compound synthesized at the site which demonstrates that characteristic.

20

A discrete sample-by-sample analysis will yield a great deal of extraneous information, as everything in the sample will be analyzed. However, in analyzing the results of a combinatorial synthesis, it is desirable to be able to track in linear terms, since all that is being tracked by a linear method is what is being added to the construct of interest in the synthesis, which will omit the presence of solvents, resin bits, side reactions and impurities.

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In view of the above needs and shortcomings of the prior art, it is a primary object of the present invention to reduce the amount of time needed to read and de-code the products of a combinatorial

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synthesis. It is another object of the present invention to provide a method of encoding combinatorial constructs that does not require orthogonal chemistry, that is, chemistry that has been carefully selected so as not to be interfering with the chemistry that is being
5 executed as part of the combinatorial synthesis itself. Another object of the invention is to minimize the amount of capital investment needed to develop a coding strategy that requires no more than what is needed to initially develop a set of appropriate solid support links.

10

Unlike the methods of the prior art, the present invention embodies a method of isotopically rather than chemically encoding a monomer to read a synthetic history. Readable differences in the encoding moieties therefore rely on physical
15 differentiation, rather than chemical differentiation. The isotopically encoded monomer is, however, chemically bonded to the ligand of interest during synthesis, in contrast to prior art identification methods in which differentiable isotopes are physically mixed into and interspersed with bulk chemicals or
20 commodities to identify their manufacturing source. The invention likewise does not rely on tagging a molecule, like other prior art approaches.

DESCRIPTION OF THE FIGURES

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Figure 1 is an array of MS peaks that represent the observed peak signatures for the doped glycine code blocks described in the specification below.

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Figure 2 is a graph that shows the observed correlation between number of carbon atoms present and the fraction of link content that has N¹⁵ present. The chart can be used to calculate equal intensity
peak signatures.

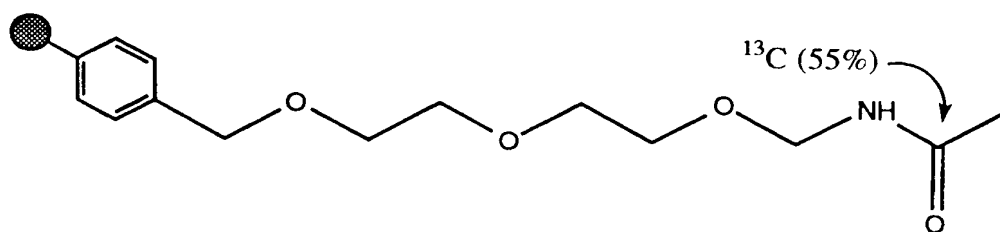
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Figure 3 illustrates a two peak positional coding strategy for a molecule, featuring splitting MS peaks into doublets to enhance code recognition.

5

Figure 4 illustrates the observed correlation between MS peak area and ratio of N¹⁵ present in a code sample for a series of code samples differing in N¹⁵ content by 5% increments.

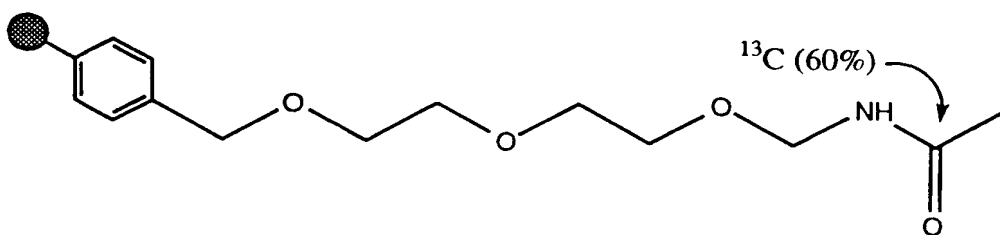
10 Figure 5 is an NMR peak pattern for the compound:



denoting that 55% of the carbon at the penultimate position was doped with C¹³.

15

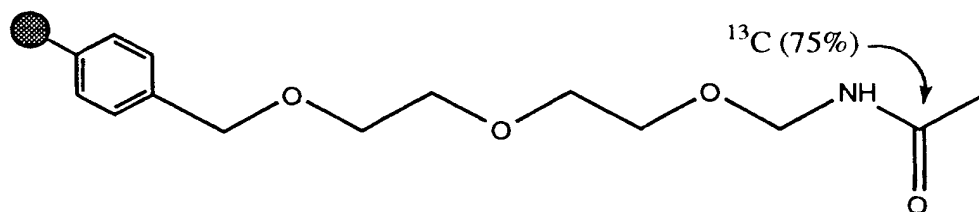
Figure 6 is an NMR peak pattern for the compound:



20 denoting that 60% of the carbon at the penultimate position was doped with C¹³.

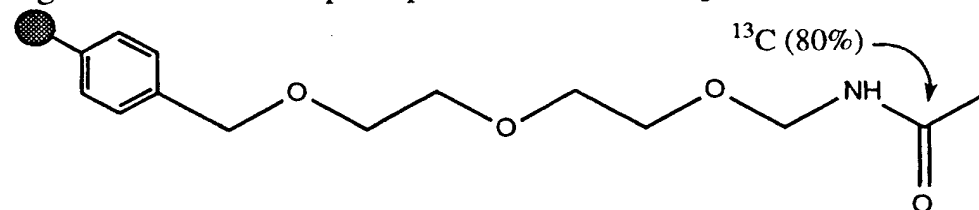
Figure 7 is an NMR peak pattern for the compound:

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denoting that 75% of the carbon at the penultimate position was doped with C^{13} .

- 5 Figure 8 is an NMR peak pattern for the compound:



denoting that 80% of the carbon at the penultimate position was doped with C^{13} .

- 10 Figure 9 is a chart that correlates the observed ratio of C^{13} to C^{12} in the NMR spectra of Figures 5 through 8 with the % of C^{13} present in each.

- 15 Figures 10 through 12 show spectra observed in conjunction with the Example 23 procedure on the Reaction Screening embodiment of the invention.

- 20 Figures 13 through 17 show synthesis flow diagrams used in the Example 23 disclosure on the Reaction Screening embodiment of the invention.

- 25 Figures 18 through 51 show spectra observed in conjunction with the Example 24 disclosure of the Ratio Coding embodiment of the invention.

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Figures 52 through 110 show spectra that are produced in conjunction the disclosure of Example 25 of encoding styrene and Example 26 disclosing copolymerization of styrene and F-styrene.

- 5 Figures 111 through 132 show spectra representing the decoding of a parallel synthesis of a library on encoded resin beads, using a dual linker approach in which a first linker was a photocleavable linker and a second linker was a Knorr linker.
- 10 Figures 133 to 286 are spectra that are produced by a set of code blocks organized in a type of ratio encoding designated as four peak ratio encoding, using four isotopically distinct F-moc protected versions of alanine
- 15 Figure 287 is a flow diagram for a computer program capable of automating the decoding of a set of code blocks and their associated spectra.

SUMMARY OF THE INVENTION

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- In brief summary, the invention is the application of mass or isotopic encoding in the imprinting of coded information into or in conjunction with materials or processes such that the details of the components or process steps can be easily determined by
- 25 one or more of the methods of mass spectrometry, nuclear magnetic resonance spectroscopy, or infrared spectroscopy, including the technique of Raman spectroscopy. In a preferred embodiment, the invention is a method of encoding the products of combinatorial synthesis by isotopically doping a portion of a
 - 30 combinatorial chemistry construct and analyzing the combinatorial reaction products by mass spectrometry, nuclear magnetic resonance spectroscopy or infrared spectroscopy.

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In overview, the invention comprises several embodiments. Firstly, the invention comprises a mass-based, non-chemical method for recording the reaction of at least a portion of a reaction series on each of a plurality of unique solid supports, the method comprising: preparing a plurality of agents each having a unique defined mass; preparing a group of unique solid supports; reacting each solid support group with a different chemical reagent under a controlled reaction condition; mixing these product groups together and then dividing the mixture of unique solid supports into a plurality of groups for a second intermediate or final stage; repeating the reacting stage with a chemical reagent under a controlled reaction condition at least once to provide a plurality of final products, having different products on the different individual unique solid supports; each of the unique defined mass agents being reacted with either: each of a group of unique solid supports; each of a group of first chemical reagents in a reaction series; each of a group of second chemical reagents in a reaction series; or each of a group of subsequently added chemical reagents in a reaction series; such that each of the group of unique solid supports, group of first chemical reagents, group of second chemical reagents or group of subsequent chemical reagents has been reacted with an agent having a defined mass that is different from any other defined mass agent reacted with any other of the aforesaid groups; the unique defined mass agents being capable of being analyzed and wherein such analysis defines the choice of a first chemical reagent, reaction condition under which the first chemical reagent was added, second chemical reagent, reaction condition under which the second chemical reagent was added, subsequent chemical reagent, or reaction condition under which the subsequent chemical reagent was added. The method is particularly preferred in performing an assay or using separation techniques including column or plate chromatography or fluorescence assistance cell scanning to find reaction products having a characteristic of interest and then

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- performing the identification analysis on such reaction products. This method is particularly preferred with respect to the determination of the first chemical reagent added to the reaction sequence. Reaction products can include a non-oligomer which is
- 5 aliphatic, alicyclic, aromatic, or heterocyclic or an oligomer which is an oligopeptide, oligonucleotide, oligosaccharide, poly lipid, polyester, polyamide, polyurethane, polyurea, polyether, polyphosphorus derivative where the phosphorus is taken from the group consisting of phosphate, phosphonate,
- 10 phosphoramidate, phosphonamide, phosphite, or phosphinamide, or polysulfur derivative where the sulfur is taken from the group consisting of sulfone, sulfonate, sulfite, sulfinamide, or sulfenamide.
- 15 Preferred means of analysis are by mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, and infrared or Raman spectroscopy. These means of analysis produce distinctive peak patterns used in analysis, identification, coding and decoding strategies. The mass spectrometer in particular
- 20 produces unique single mass peaks, unique double mass peaks, unique pairs of single mass peaks, unique pairs of double mass peaks and other unique multiple peak patterns, which are additionally capable of being translated into machine-readable patterns, including bar codes. The NMR, IR and Raman methods
- 25 also produce useful unique peak patterns that can be transformed into machine-readable patterns, including bar codes. Such patterns, including machine-readable patterns, can be assigned in encoding strategies to represent discrete chemical reagents added in a chemical reaction series, or assigned to represent discrete
- 30 chemical reaction conditions under which a chemical reagent was added in a reaction series, including, but not limited to, concentrations, temperatures, pressures, catalysts, enzymes, electromagnetic energy imparted to the system (visible light conditions, irradiation, etc.), and so on. This is done by initially

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generating recognition patterns for unique mass defined agents which are either eye-readable or machine-readable. The patterns resulting from the analysis steps are then compared to the recognition patterns for identification and decoding, with or
5 without the assistance of a machine. Recognition patterns and analysis patterns that are machine-readable are capable of being stored electronically in suitable computer storage and memory devices well known to those of ordinary skill in the art and such stored patterns form a readily retrievable database that also forms
10 a claimed embodiment of the invention.

Mass agents used in these methods are preferably a molecular entity such as a solid state chemistry support resin bead linker which has had one or more of its atoms replaced by an
15 isotope of that atom, thus altering the mass, but not the chemical properties of that chemical entity. Another alternative mass agent is to simply repeat the appearance of such a molecular entity an integral number of times in a linear chemical construct.

20 The method of the invention can be performed using solid state chemistry or solution chemistry. Reaction products can be cleaved off of a solid support for analysis, or the entire construct can be analyzed. Chemical reagents added to a reaction series can include one or more that can act as a substrate for the
25 determination of binding specificity to a chemical compound of interest. Any or all of the steps of encoding, synthesis, analysis and decoding can be automated with the aid of suitable computer means and robotics means, all by methodologies well known to those of ordinary skill in the combinatorial chemistry,
30 spectroscopy, computer science, robotics and optical scanning arts.

The invention also comprises a kit made up of a set of compounds prepared for the encoding, synthesis, analysis and

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decoding methods described above of chemical reagents and reaction conditions in chemical reactions of interest in a combinatorial synthesis, where the compounds in the kit have different distinguishable masses, but the same chemical properties.

- 5 Such kits can comprise solid state supports attached to isotopically doped linkers, series or combinations of isotope mixtures in readily identifiable ratios, or a variety of combinations of isotopically doped chemical moieties (also called code blocks herein) with covalent bonds or other organic moieties as links
10 or linkers.

- The invention also comprises kits of solid state supports that have been prepared so as to bear unique defined mass agents on their surface. Solid supports can be resin beads in a wide range of
15 sizes of from 1 to 10,000 μm in dimension. The support itself can be isotopically doped by incorporating an isotope into the chemical structure of the support. This differentiates the mass of the support from like supports, but does not change its chemical properties. Such solid supports would then be used in the methods
20 of the invention. Groups of such uniquely mass-defined supports can comprise libraries, which are also an embodiment of the invention as claimed herein.

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DETAILED DESCRIPTION OF THE PREFERRED
EMBODIMENTS AND BEST MODE OF THE INVENTION

5 The term "isotopic doping" or "isotopically doped" is used here to mean the introduction into a molecule of an isotope of one or more of the atoms normally appearing in that molecule.

10 A "link" is a covalent bond or a molecular moiety that is suitable for linking two portions of a construct together.

15 A "linker" is a moiety comprised of a code block, a first link for bonding the code block to a solid support or other moiety, and a second link for bonding the code block to a ligand or other moiety.

20 A "linker mix" is a mixture of two isotopically distinct but chemically identical molecules, the mix having a discrete ratio of first isotope to second isotope.

25 A "code block" is a molecule or series of molecules containing one or more isotopically doped atoms or having a repeating number of molecules in series, such that its mass can be discretely defined and differentiated from any other code block.

30 A "doped" atom, code block or moiety refers to an entity in which an atom in a molecule which has been replaced by one of its isotopes. Doped atoms as contemplated by this invention include all atoms found in organic chemistry having isotopes and in particular, hydrogen, carbon, nitrogen, fluorine and oxygen. Non-radioactive isotopes are generally preferred over radioactive ones.

 The term "monomer" is used herein not only to denote subunits that make up a linear molecules such as amino

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acids, but also to denote starting chemical materials and chemical reagents that are chemically reacted with one another in combinatorial chemistry organic synthesis to produce molecules that are not linear, such products including so-called "small
5 molecules" which are organic molecules of 500 Daltons or less in molecular weight.. As used herein, the terms "monomer" and "chemical reagent" are interchangeable.

10 A "reaction series" as used herein refers to a series of steps in a chemical synthesis in a combinatorial synthesis format.

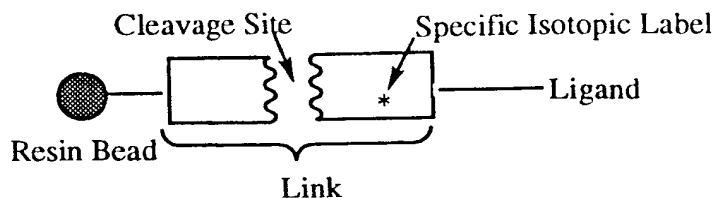
A "solid support" is one or materials upon which combinatorial chemistry syntheses can be performed, including beads, solid surfaces, solid substrates, particles, pellets, disks,
15 capillaries, hollow fibers, needles, solid fibers, cellulose beads, pore-glass beads, silica gels, polystyrene beads optionally crosslinked with divinylbenzene, grafted co-polybeads, polyacrylamide beads, latex beads, dimethylacrylamide beads optionally cross-linked with N,N'-bis-acryloyl ethylene diamine,
20 glass particles coated with a hydrophobic polymer, fullerenes and soluble supports such as low molecular weight non-cross-linked polystyrene.

25 A "construct" is a covalently bonded entity comprising, in any combination, some type of solid support, one or more linkers, one or more code blocks, and one or more ligands.

30 A "ligand" is a chemical reaction product of interest. A ligand can be part of a larger construct, where the ultimate goal will be to identify and/or cleave the ligand apart from the rest of the construct.

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In general, the methods of the invention make use of some variant of a construct of the non-limiting general formula:



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Isotopes are inserted into such constructs via doped links. That is, after the determination has been made as to what will constitute a chemically suitable link, the link is synthesized using one or more atoms of the link that has been substituted by an isotopic form of that atom. The isotopic form is chemically indistinguishable from the non-isotopic form of the link. The isotopic form is, however physically distinguishable from other isotopic forms by MS or NMR analysis. This physical difference is manifested by differently appearing MS, IR, or NMR peak patterns. A wide variety of isotopic insertion or doping strategies are available, leading to a large number of physically distinguishable chemical moieties, each having its own distinctive peak pattern. The existence of such distinctive peak patterns means that the isotopic forms can be used to create a code that can be used in various encoding strategies to readily identify different chemical entities or conditions that can be used during a combinatorial synthesis. Several of such encoding strategies are given below.

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Electrospray mass spectrometry provides mass and ionic charge intensity information for a given molecular entity. In general, smaller molecules only appear in the spectrum once as singly charged species, and the effect of isotopic compositional variation results in a very predictable quantum mass shift. Thus, for example, a molecule having a molecular weight of 304 will produce a peak at 305 ($M + 1$) on a mass spectrometer (MS) spectrum. If a nitrogen atom in such molecule having an atomic

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weight of 14 (N^{14}) is replaced (by isotopic doping, as that term is used herein) by isotope N^{15} , then the MS peak will shift to the right precisely one unit to 306. Furthermore, such isotopic doping will not affect the ionization or chemical reactivity of the molecule. The ability to measure both of the properties of mass and ionic intensity with reasonable accuracy (i.e. mass to about 0.1 atomic mass units and relative intensity to about 3% provides the basis for a novel encoding strategy using isotopes to isotopically, rather than chemically, encode a monomer to read a synthetic history, instead of tagging a molecule itself. Using the methods of the invention, encoding strategies are devised from the use of the mass information alone, the relative intensity information in two or more mass peaks alone or a combination of the two. The basic methodology of the invention, which is to insert different isotopes into combinatorial constructs to identify addition of monomers or chemical conditions, gives rise to several alternative embodiments for encoding and decoding, which are capable of individual implementation or in selected combinations.

Multiple preferred coding approaches using defined mass agents will be discussed. These examples are non-limiting and other coding approaches are not meant to be excluded.

Example 1: Encoding Approaches

Encoding Example A: Ratio Coding Approach

In a most preferred embodiment of the invention, a series of isotopically doped linker mixes n is prepared, with each linker mix in the series comprising a discrete ratio of first isotope to second isotope, and with each mix in the series differing in ratio from the previous or subsequent mix in the series by an equal amount. Thus, for example, a series of 21 batches of doped

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monomer can be prepared, with the first batch in the series having a ratio of 100% of first isotope to 0% of second isotope, the second batch having a ratio of 95% of first isotope to 5% of second isotope, and so on until the 21st batch, which will have 0% of first isotope and 100% of second isotope. Each linker is further characterized by being capable of being cleaved such that one cleavage portion will always contain the isotopically doped molecular moiety. Each linker mix is chemically reacted with a support means on one side of the linker to yield n batches of support-linker, again each having a discrete ratio of first to second isotope. Then a reagent of interest is added to each batch to yield n batches of support-linker-molecule, each likewise having the discrete ratios of two isotopes. If, now for example, a split synthesis method is being pursued, then the n batches are combined into a single mixing vessel and the resulting mix is then divided into n aliquots, and to each aliquot is added a second reagent of interest, and the resultant reactions are allowed to proceed. The previous step of combining all aliquots and then splitting them out into n aliquots is repeated, and a third reagent of interest can be added. If all possible reactions have proceeded to completion, then there are now n^3 products. The linkers can be cleaved so that the isotopically labeled fragment stays with the chemical product, and the resulting isotope label-chemical product construct is analyzed by a mass spectrometer. The resulting mass spectrogram will display a peak pattern that mirrors the ratio of first isotope to second isotope present in the linker fragment, thus identifying what the first chemical reagent was that was added to that particular reaction well. Thus, for example, if the isotope ratio is 100% for first isotope to 0% for second isotope, then one relatively tall peak will appear on the spectrogram at the position on the spectrum occupied by the discrete atomic mass that that moiety has (as will be appreciated by those of ordinary skill in the art, other relatively small peaks, will, of course appear, but just one prominent peak that is characteristic of the molecule as used

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in the identification method of the present invention appears.). If the isotope ratio is 95% of first isotope to 5% of second isotope, then there will be a first prominent peak and a second prominent peak, with the first prominent peak having about 95% of the total area under the combined prominent peaks, and the second prominent peak having the remaining approximately 5%, thus forming a distinct prominent peak pattern which serves to clearly identify the isotope ratio and therefore the chemical reagent. It is to be understood that another smaller peak not part of the patterns forming the codes of the invention will appear, by virtue of the natural abundance of C^{13} , and its relative size can be predicted using the following analysis. If a given moiety contains 24 carbons and nitrogen and it has been doped to a 50/50 ratio of N^{14}/N^{15} , then peak 1 will be calculated from the N^{14}/C^{12} present as $(0.898)^{24} \times 0.5 = 0.383$; peak 2 will be calculated from the N^{15}/C^{12} and N^{14}/C^{13} present as $(0.989)^{24} \times 0.5 + (0.989)^{23} (0.011) \times 24 \times 0.5 = 0.485$; and the third peak will be calculated from the N^{15}/C^{13} present as $(0.989)^{23} \times (0.011) \times 24 \times 0.5 = 0.102$, thus resulting in a distinctive pattern having two approximately equal prominent peaks, and the relatively small side peak. Ideally, the isotopically distinct species used should differ by two or more AMUs as this would produce well separated peaks that would not be affected by the natural presence of ^{13}C . To achieve this with the atoms that are most commonly used in such chemistry (C,H,N,O) typically requires that more than one atom be isotopically substituted, as available stable isotopes of C, H and N differ by only one mass unit.

Subsequently, in order to identify the third component in the combinatorial construct, the third component can be identified by its reaction well number. Now, with the identity of the first component known by virtue of the mass spectrograph and the third component known by virtue of the well number, their combined molecular weights are subtracted from the total

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molecular weight of the construct to arrive at a residual mass which identifies the second component of the construct.

5 It should be appreciated that using the methods and constructs of the invention provide for an entire series of encoded linkers that provide great economic savings, since the only additional synthesis initially required is to isotopically dope a solid support linker that had to be constructed in the first place. No other chemical modifications are needed to be made to the linkers
10 that were initially chosen for the solid state combinatorial synthesis.

Encoding Example B: Dual Ratio Coding Approach

15 For syntheses having more than three steps, the procedure of Ratio Coding described above is followed in that a series of isotopically doped linker mixes n is prepared, with each linker mix in the series comprising a discrete ratio of first isotope to second isotope, and with each mix in the series differing in ratio
20 from the previous or subsequent mix in the series by an equal amount. However, a second type of atom is also isotopically doped, so that there are two types of atomic isotopes present. For example a nitrogen position can be doped with a mix of N^{14} and N^{15} , while a carbon position can be doped with a mix of C^{12} and
25 C^{13} . Again, this example will follow the progression of 5% increments, so that there are 21 possible nitrogen ratio combinations and 21 possible carbon ratio combinations. Furthermore, the carbon position is protected with a suitable protecting group, which can be deprotected to correlate to a
30 reaction step in the combinatorial synthesis. Thus, the mass spectrograph peak pattern for the doped nitrogen identifies the first synthesis component, the mass spectrograph peak pattern for the doped carbon identifies the second synthesis component, an assay identifies the fourth component, and the third component is

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identified by subtraction of the first, second and fourth components from the total weight of the product.

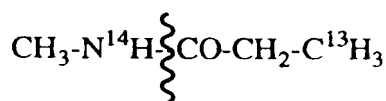
- 5 Occasionally, code degeneration may arise when using more than one type of isotope, as in the case where one moiety bore a N¹⁵-C¹² combination and another bore a N¹⁴-C¹³ combination, both of which would have the same atomic weight and therefore give indistinguishable peak patterns in a mass spectrograph. This
 10 problem can be circumvented in one of two general ways. Firstly, by selecting C¹² and C¹⁴ as the carbon isotope pair, this type of code degeneracy is avoided. Or, if radioactivity is to be avoided, C¹² and C¹³ can still be used by the technique of combining mass spectrography with fragmentation. For example, an amide moiety
 15 of the structure



- 20 can be doped in two ways that will yield the same atomic weight as follows:

I

II



or

25

- However, when they are fragmented along the indicated cleavage lines, moiety I will yield a fragment having a doped N and a normal C, while moiety II will yield a fragment having a doped C and a normal N, thereby yielding distinctive peak patterns in the
 30 MS.

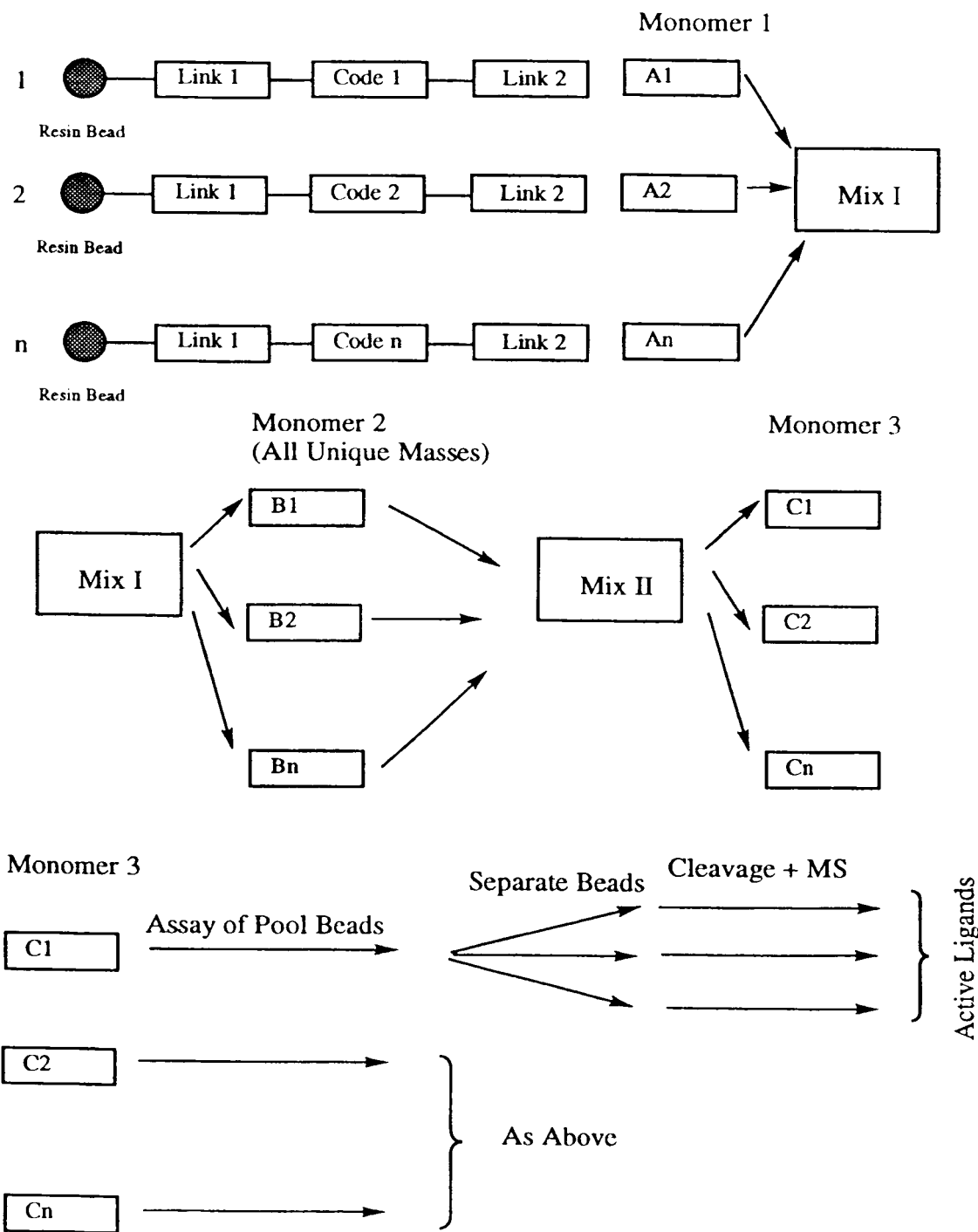
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The following scheme illustrates the Single Peak Positional Coding Approach

Encoding Example C: Single Peak Positional Coding

5

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Scheme 2

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Following the process in Scheme 2, another alternative embodiment of the invention of isotopic encoding can follow a single MS peak position approach. This approach generates a MS peak. In this format, the mass of the coding block is engineered
5 such that it appears in a convenient part of the spectrum, and it is used to represent the first monomer or building block used in the synthesis of a particular compound. After the combinatorial synthesis is complete, Link 2 is cleaved in order to determine the molecular weight of the ligand. Then Link 1 is cleaved to enable
10 identification of the Code block, which determines the identification of the first pool monomer.

The first and second links in a linker scheme can be the same or different. The links can be the same when a direct binding assay is
15 used, followed by removal of positive beads. When the links are different, this allows the assay to be either by direct binding or with cleaved ligand alone.

Decoding of a three monomer combinatorial library is achieved
20 from three steps. The mass of the code block is determined, thus identifying the code, which identifies the first pool monomer. The pool number of the final monomer addition determines the final monomer, from which the mass of the final monomer is known. Then, the masses of the first and third pool monomers are
25 subtracted from the total molecular weight of the entire construct, thus leaving the mass of the second pool monomer, from which the identity of the second pool monomer is inferred.

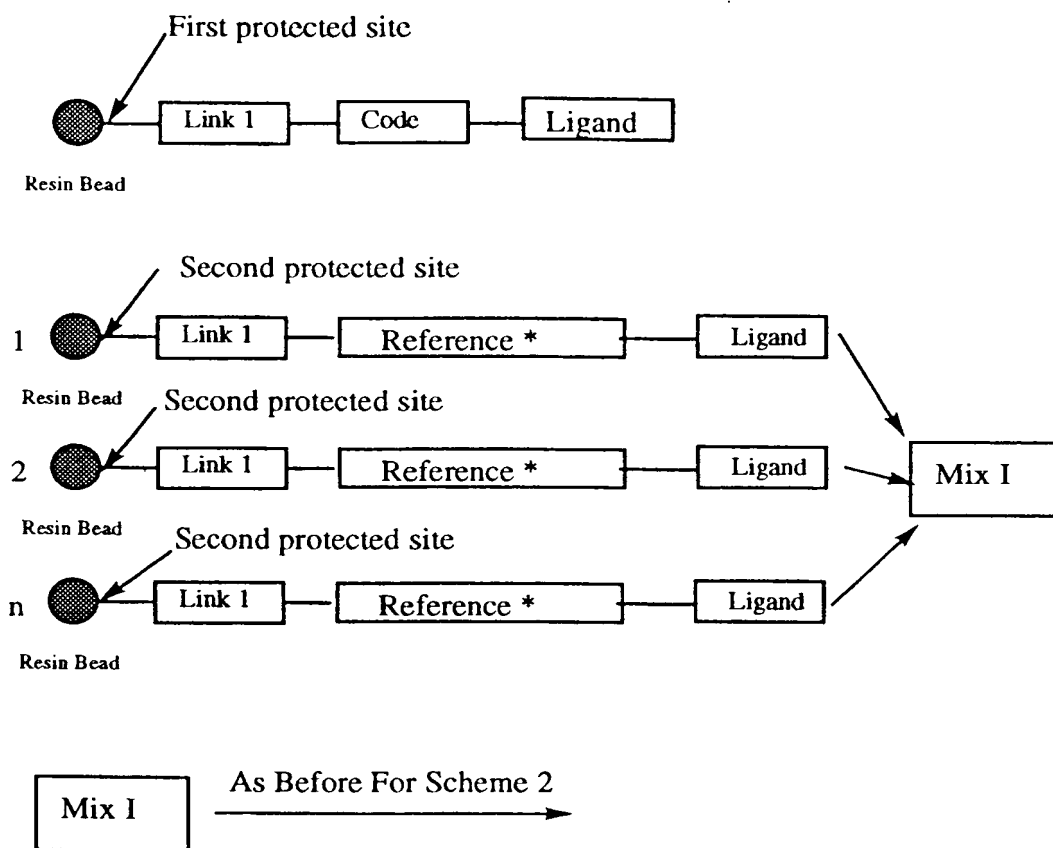
The Single Peak Positional encoding approach is especially useful
30 when active ligands are screened by use of a chromatography column, and the bound constructs are then eluted.

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The following scheme illustrates the Double Peak Positional Coding Approach

Encoding Example D: Double Peak Positional Coding

5



Scheme 3

Following the process in Scheme 3, another alternative
 10 embodiment of the invention of isotopic encoding can follow a
 double MS peak position strategy. This approach yields two MS
 peaks. For example, a set of resin beads is prepared wherein the
 beads bear two protected sites, such as BOC and FMOC. A first
 protected site is de-protected. A number of isotopic code blocks n
 15 is prepared and each code is linked to a first de-protected site.

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The second protected site is de-protected. A standard reference moiety having a different mass is linked to the second de-protected site. Upon MS, this will yield a peak for the standard reference and a second peak for the code, with the mass difference between the two peaks being the encoding unit.

5 Decoding of a three monomer combinatorial library is achieved from three steps. The mass of the ligand plus code block is determined, thus identifying the code, which identifies the first pool monomer. The pool number of the final monomer addition

10 determines the final monomer, from which the mass of the final monomer is known. Then, the masses of the first and third pool monomers are subtracted from the total molecular weight of the entire construct, thus leaving the mass of the second pool monomer, from which the identity of the second pool monomer is

15 inferred.

In an alternative construct, the code block is prepared to contain two like species, one of which is constant and acts as a reference mass, while the other has variable mass blocks similar to those

20 described for the single peak positional encoding scheme. After cleavage of the link to the resin, ligand is generated with an attached variable code increment. The encoded information is now determined from the difference in mass of the coding unit and the reference mass unit. The advantage of this is that the code

25 can be left attached to the ligand and read at the same time as the mass of the whole construct is determined by MS. This strategy gives two mass peaks that represent the ligand attached to either the reference code or the variable code. The difference in mass is used to determine the identity of monomer 1, while monomer 3

30 known from the corresponding pool and therefore monomer 2 is inferred as described above.

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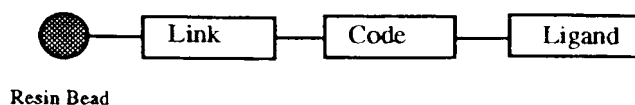
The invention also makes possible an alternative embodiment that in effect creates a bar coding mechanism. Several non-limiting exemplary versions of solid state constructs are possible as follows.

5

Encoding Example E: The Bar Coding Approach

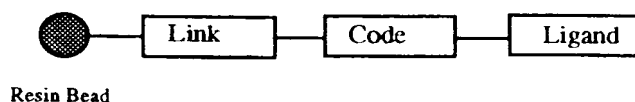
Version 1: Mass only:

10



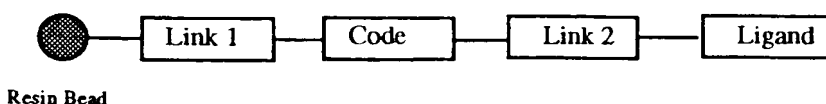
Version 2: Mass and Intensity:

15

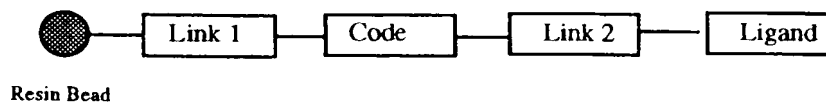


Version 3: Mass only:

20



Version 4: Mass and Intensity:



25

In this approach and its variations, a code block most advantageously consists of a dimer, each of which unit has one, two or three discrete masses. For example a construct of the structure:

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contains a code block having two mass blocks, M₁ and M₂, which in this example will be comprised of one, two or three amino acids. The amino acids are doped to give three discrete masses yielding, for example, Glycine (G⁰), with a mass of 57, Glycine bearing N¹⁵ (G¹) with a mass of 58 and Glycine bearing two Deuterium atoms (G²) at carbon 2 with a mass of 59. Assembling these three moieties into 7 possible mass sets yields the following codes and their fractions of the total:

<u>Composition</u>	<u>Relative Presence</u>
G ⁰	100%
G ¹	100%
G ²	100%
G ⁰ G ¹	50% and 50%
G ⁰ G ²	50% and 50%
G ¹ G ²	50% and 50%
G ⁰ G ¹ G ²	33.3%, 33.3% and 33.3%

It is now possible to assemble these mass sets into blocks M₁ and M₂ and to predict what the characteristic MS peak pattern will be for each code block. For this group of mass sets in Version 1 of the Bar Coding Approach, the following MS peak pattern is predicted:

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<u>Code</u> <u>Sample</u>	<u>M1</u>	<u>M2</u>	<u>Predicted Peak</u> <u>Pattern, (AMU)</u>				
			1	1	1	1	1
			1	1	1	1	1
			4	5	6	7	8
1	57 (G ⁰)	57 (G ⁰)	■				
2	57 (G ⁰)	57,58 (G ⁰),(G ¹)	■	■			
3	57 (G ⁰)	57,59 (G ⁰),(G ²)	■		■		
4	57 (G ⁰)	57,58,59 (G ⁰),(G ¹),(G ²)	■	■	■		
5	57,58 (G ⁰),(G ¹)	57,59 (G ⁰),(G ²)	■	■	■	■	
6	57,59 (G ⁰),(G ²)	57,59 (G ⁰),(G ²)	■		■		■
7	57,59 (G ⁰),(G ²)	57,58,59 (G ⁰),(G ¹),(G ²)	■	■	■	■	■

In this table, for code sample 1 a single peak is predicted at 114, since the combined masses of each G⁰ are 57 + 57 = 114. For code sample 2, two equal peaks are predicted at 114 and 115, since G⁰ in M1 plus G⁰ in M2 adds up to 114, and G⁰ in M1 plus G¹ in M2 adds up to 115. In code sample 6, two single peaks are predicted at 114 and 118 and a redundancy is predicted at 116 to yield a peak approximately two times as tall as the peaks at 114 and 118.

10

In the following table, predicted MS peak patterns are shown for 25 code samples. The actual MS peak patterns are shown below

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- the predicted peak pattern table, and it can be seen that there is excellent correlation between observed and predicted peak patterns. References to G⁰, G¹ and G² are omitted although the same three isotopically doped versions of Glycine described above
- 5 are still being used in this table (Note that the numerical values for the MS in Figure 1 are for the total mass of the entire construct, including a Lysine residue and the linkers, which fall into a range of 300 to 309 mass units)

<u>Code</u> <u>Sample</u>	<u>M1</u>	<u>M2</u>	<u>Predicted Peak</u> <u>Pattern (AMU)</u>				
			1	1	1	1	1
			1	1	1	1	1
			4	5	6	7	8
1	57	57	■				
2	57	58		■			
3	57	57,58	■	■			
4	57	59			■		
5	57	57,59	■		■		
6	57	58,59		■	■		
7	57	57,58,59	■	■	■		
8	58	59					■
9	58	57, 59		■			■

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10	59	57, 58			■	■
11	58	57, 58, 59		■	■	■
12	57, 58	57, 58			■	
				■	■	■
13	57, 58	57, 59	■	■	■	■
14	57, 58	58, 59				■
					■	■
						■
15	57, 58	57, 58, 59		■	■	
			■	■	■	■
16	59	59				■
17	59	57, 59			■	■
18	57, 59	57, 59			■	
			■		■	■
19	59	58, 59				■
						■
20	59	57, 58, 59			■	■
					■	■
21	57, 59	58, 59		■	■	■
					■	■
22	57, 59	57, 58, 59			■	
			■	■	■	■
						■
23	58, 59	58, 59			■	■
					■	■

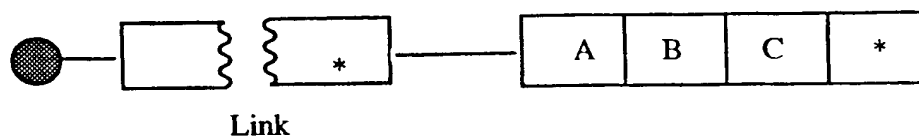
-33-

24	58, 59	57, 58, 59							
25	57, 58, 59	57, 58, 59							

- Once the MS is run on a completed combinatorial synthesis sample, the pattern is compared to a predicted pattern, which in turn identifies the code, which in turn identifies the first pool
- 5 monomer. The predicted patterns can form a type of machine readable bar code, which, using methods well known to those of ordinary skill in the optical scanning art, enables rapid data inputting, decoding and interpretation.

10 Other Encoding Strategies

- It is possible to combine elements of one or all of the ratio, single peak, double peak or bar code methods to give code constructs. Code blocks don't have to be added into the front end
- 15 of a construct-they can be added in at any point, assuming a suitable reagent is available, for example:

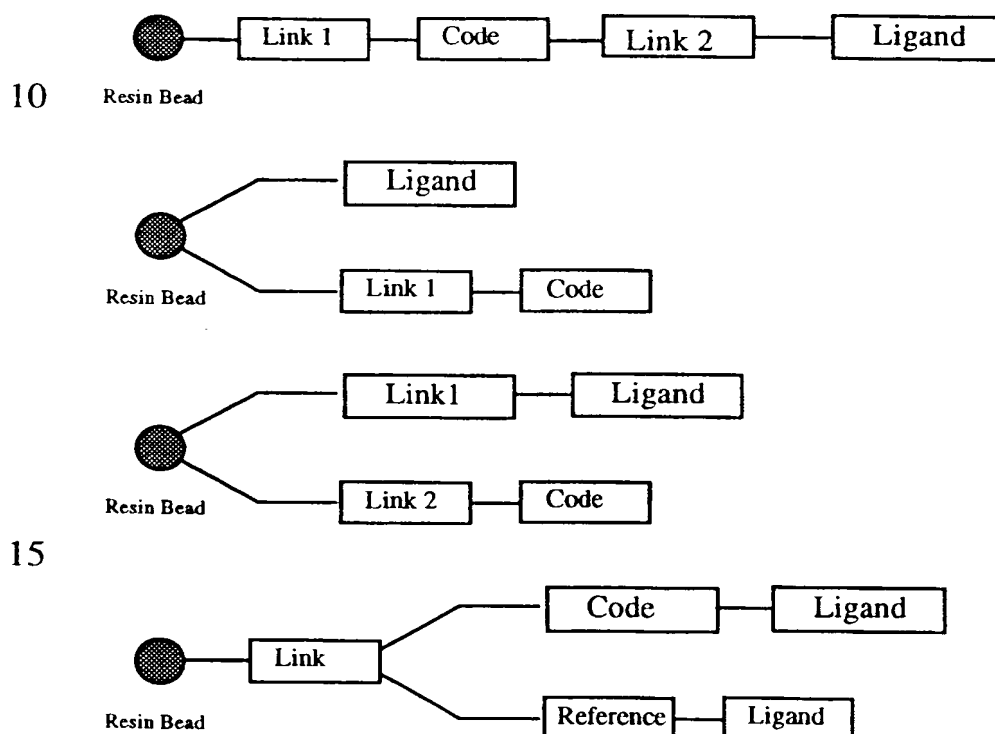


- 20 where isotope doping occurs at the bead link and attached to the end of a three monomer ligand, as indicated by the asterisk. Isotope doping could also be performed at the other end of the ligand as well.
- 25 In another alternative embodiment of the invention, since isotopes can be readily read by nuclear magnetic resonance

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(NMR), this can be used instead of, or in conjunction with, MS spectroscopy. NMR offers the advantages of greater accuracy (one can run ratio pools in increments of down to 1%), the bead does not have to be cleaved off of the construct prior to analysis, and that NMR, unlike MS, is non-destructive.

Other constructs can take the general non-limiting exemplary formulae:



20 Figures 133 to 286 display spectragrams associated with another variation of ratio encoding, which is a four peak ratio encoding scheme. In the following table, the first column represents the code block, the second column is an internal reference number, and the third, fourth, fifth and sixth columns represent the respective mole fractions of Fmoc-protected alanines with different integral masses using isotope doping, i.e.

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A^0 , A^3 , A^2 , and A^1 (A is reference alanine, A^3 is alanine plus 3 atomic mass units, A^2 is alanine plus two atomic mass units, etc.).

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1	,111,	0.56,	0.16,	0.16,	0.16
2	,112,	0.56,	0.16,	0.16,	0.21
3	,113,	0.51,	0.11,	0.11,	0.26
4	,114,	0.46,	0.11,	0.11,	0.31
5	,115,	0.46,	0.11,	0.11,	0.36
6	,116,	0.41,	0.11,	0.11,	0.41
7	,121,	0.56,	0.16,	0.21,	0.16
8	,122,	0.51,	0.11,	0.21,	0.21
9	,123,	0.46,	0.11,	0.21,	0.26
10	,124,	0.46,	0.11,	0.16,	0.31
11	,125,	0.41,	0.11,	0.16,	0.36
12	,126,	0.41,	0.11,	0.16,	0.41
13	,131,	0.51,	0.11,	0.26,	0.11
14	,132,	0.46,	0.11,	0.26,	0.21
15	,133,	0.46,	0.11,	0.26,	0.26
16	,134,	0.41,	0.11,	0.21,	0.31
17	,135,	0.41,	0.11,	0.21,	0.31
18	,136,	0.36,	0.11,	0.21,	0.36
19	,141,	0.46,	0.11,	0.31,	0.11
20	,142,	0.46,	0.11,	0.31,	0.16
21	,143,	0.41,	0.11,	0.31,	0.21
22	,144,	0.41,	0.11,	0.26,	0.26
23	,145,	0.36,	0.11,	0.26,	0.31
24	,146,	0.36,	0.11,	0.26,	0.36
25	,151,	0.46,	0.11,	0.36,	0.11
26	,152,	0.41,	0.11,	0.36,	0.16
27	,153,	0.41,	0.11,	0.31,	0.21
28	,154,	0.36,	0.11,	0.31,	0.26
29	,155,	0.36,	0.11,	0.31,	0.31
30	,156,	0.31,	0.11,	0.26,	0.31
31	,161,	0.41,	0.11,	0.41,	0.11
32	,162,	0.41,	0.11,	0.41,	0.16
33	,163,	0.36,	0.11,	0.36,	0.21
34	,164,	0.36,	0.11,	0.36,	0.26
35	,165,	0.31,	0.11,	0.31,	0.26
36	,166,	0.31,	0.11,	0.31,	0.31
37	,211,	0.56,	0.21,	0.16,	0.16
38	,212,	0.51,	0.21,	0.11,	0.21
39	,213,	0.46,	0.21,	0.11,	0.26
40	,214,	0.46,	0.16,	0.11,	0.31
41	,215,	0.41,	0.16,	0.11,	0.36
42	,216,	0.41,	0.16,	0.11,	0.41
43	,221,	0.51,	0.21,	0.21,	0.11
44	,222,	0.46,	0.21,	0.21,	0.21
45	,223,	0.46,	0.16,	0.16,	0.26
46	,224,	0.41,	0.16,	0.16,	0.31
47	,225,	0.41,	0.16,	0.16,	0.31
48	,226,	0.36,	0.16,	0.16,	0.36
49	,231,	0.46,	0.21,	0.26,	0.11
50	,232,	0.46,	0.16,	0.26,	0.16

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51	,233,	0.41,	0.16,	0.21,	0.21
52	,234,	0.41,	0.16,	0.21,	0.26
53	,235,	0.36,	0.16,	0.21,	0.31
54	,236,	0.36,	0.16,	0.21,	0.36
55	,241,	0.46,	0.16,	0.31,	0.11
56	,242,	0.41,	0.16,	0.31,	0.16
57	,243,	0.41,	0.16,	0.26,	0.21
58	,244,	0.36,	0.16,	0.26,	0.26
59	,245,	0.36,	0.16,	0.26,	0.31
60	,246,	0.31,	0.16,	0.26,	0.31
61	,251,	0.41,	0.16,	0.36,	0.11
62	,252,	0.41,	0.16,	0.31,	0.16
63	,253,	0.36,	0.16,	0.31,	0.21
64	,254,	0.36,	0.16,	0.31,	0.26
65	,255,	0.31,	0.16,	0.26,	0.26
66	,256,	0.31,	0.11,	0.26,	0.31
67	,261,	0.41,	0.16,	0.41,	0.11
68	,262,	0.36,	0.16,	0.36,	0.16
69	,263,	0.36,	0.16,	0.36,	0.21
70	,264,	0.31,	0.16,	0.31,	0.26
71	,265,	0.31,	0.11,	0.31,	0.26
72	,266,	0.31,	0.11,	0.31,	0.31
73	,311,	0.51,	0.26,	0.11,	0.11
74	,312,	0.46,	0.26,	0.11,	0.21
75	,313,	0.46,	0.26,	0.11,	0.26
76	,314,	0.41,	0.21,	0.11,	0.31
77	,315,	0.41,	0.21,	0.11,	0.31
78	,316,	0.36,	0.21,	0.11,	0.36
79	,321,	0.46,	0.26,	0.21,	0.11
80	,322,	0.46,	0.26,	0.16,	0.16
81	,323,	0.41,	0.21,	0.16,	0.21
82	,324,	0.41,	0.21,	0.16,	0.26
83	,325,	0.36,	0.21,	0.16,	0.31
84	,326,	0.36,	0.21,	0.16,	0.36
85	,331,	0.46,	0.26,	0.26,	0.11
86	,332,	0.41,	0.21,	0.21,	0.16
87	,333,	0.41,	0.21,	0.21,	0.21
88	,334,	0.36,	0.21,	0.21,	0.26
89	,335,	0.36,	0.21,	0.21,	0.31
90	,336,	0.31,	0.21,	0.21,	0.31
91	,341,	0.41,	0.21,	0.31,	0.11
92	,342,	0.41,	0.21,	0.26,	0.16
93	,343,	0.36,	0.21,	0.26,	0.21
94	,344,	0.36,	0.21,	0.26,	0.26
95	,345,	0.31,	0.21,	0.26,	0.26
96	,346,	0.31,	0.16,	0.21,	0.31
97	,351,	0.41,	0.21,	0.31,	0.11
98	,352,	0.36,	0.21,	0.31,	0.16
99	,353,	0.36,	0.21,	0.31,	0.21
100	,354,	0.31,	0.21,	0.26,	0.26

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101	,355,	0.31,	0.16,	0.26,	0.26
102	,356,	0.31,	0.16,	0.26,	0.31
103	,361,	0.36,	0.21,	0.36,	0.11
104	,362,	0.36,	0.21,	0.36,	0.16
105	,363,	0.31,	0.21,	0.31,	0.21
106	,364,	0.31,	0.16,	0.31,	0.21
107	,365,	0.31,	0.16,	0.31,	0.26
108	,366,	0.31,	0.16,	0.31,	0.31
109	,411,	0.46,	0.31,	0.11,	0.11
110	,412,	0.46,	0.31,	0.11,	0.16
111	,413,	0.41,	0.31,	0.11,	0.21
112	,414,	0.41,	0.26,	0.11,	0.26
113	,415,	0.36,	0.26,	0.11,	0.31
114	,416,	0.36,	0.26,	0.11,	0.36
115	,421,	0.46,	0.31,	0.16,	0.11
116	,422,	0.41,	0.31,	0.16,	0.16
117	,423,	0.41,	0.26,	0.16,	0.21
118	,424,	0.36,	0.26,	0.16,	0.26
119	,425,	0.36,	0.26,	0.16,	0.31
120	,426,	0.31,	0.26,	0.16,	0.31

121	,431,	0.41,	0.31,	0.21,	0.11
122	,432,	0.41,	0.26,	0.21,	0.16
123	,433,	0.36,	0.26,	0.21,	0.21
124	,434,	0.36,	0.26,	0.21,	0.26
125	,435,	0.31,	0.26,	0.21,	0.26
126	,436,	0.31,	0.21,	0.16,	0.31
127	,441,	0.41,	0.26,	0.26,	0.11
128	,442,	0.36,	0.26,	0.26,	0.16
129	,443,	0.36,	0.26,	0.26,	0.21
130	,444,	0.31,	0.26,	0.26,	0.26
131	,445,	0.31,	0.21,	0.21,	0.26
132	,446,	0.31,	0.21,	0.21,	0.31
133	,451,	0.36,	0.26,	0.31,	0.11
134	,452,	0.36,	0.26,	0.31,	0.16
135	,453,	0.31,	0.26,	0.26,	0.21
136	,454,	0.31,	0.21,	0.26,	0.21
137	,455,	0.31,	0.21,	0.26,	0.26
138	,456,	0.31,	0.21,	0.26,	0.31
139	,461,	0.36,	0.26,	0.36,	0.11
140	,462,	0.31,	0.26,	0.31,	0.16
141	,463,	0.31,	0.21,	0.31,	0.16
142	,464,	0.31,	0.21,	0.31,	0.21
143	,465,	0.31,	0.21,	0.31,	0.26
144	,466,	0.26,	0.21,	0.26,	0.26

-39-

In the following table, each of the enumerated code blocks listed immediately above is correlated with a spectrogram number identified in the second column and shown on the face of each respective spectra in the figures.

5

Code Block	Spectra Identifying Number
1	ASA008683
2	ASA 008684
3	ASA 008685
4	ASA 008686
5	ASA 008687
6	ASA 008688
7	ASA 008689
8	ASA 008690
9	ASA 008691
10	ASA 008692
11	ASA 008693
12	ASA 008694
13	ASA 008695
14	ASA 008696
15	ASA 008697
16	ASA 008698
17	ASA 008699
18	ASA 008700
19	ASA 008701
20	ASA 008702
21	ASA 008703
22	ASA 008704
23	ASA 008705
24	ASA 008706
25	ASA 008707
26	ASA 008708

-40-

27	ASA 008709
28	ASA 008710
29	ASA 008711
30	ASA 008712
31	ASA 008713
32	ASA 008714
33	ASA 008715
34	ASA 008716
35	ASA 008717
36	ASA 008718
37	ASA 008719
38	ASA 008720
39	ASA 008721
40	ASA 008722
41	ASA 008723
42	ASA 008724
43	ASA 008725
44	ASA 008726
45	ASA 008727
46	ASA 008728
47	ASA 008729
48	ASA 008730
49	ASA 008731
50	ASA 008732
51	ASA 008733
52	ASA 008734
53	ASA 008735
54	ASA 008736
55	ASA 008737
56	ASA 008738
57	ASA 008739
58	ASA 008740
59	ASA 008741
60	ASA 008742

-41-

61	ASA 008743
62	ASA 008744
63	ASA 008745
64	ASA 008746
65	ASA 008747
66	ASA 008748
67	ASA 008749
68	ASA 008750
69	ASA 008751
70	ASA 008752
71	ASA 008753
72	ASA 008754
73	ASA 008755
74	ASA 008756
75	ASA 008757
76	ASA 008758
77	ASA 008759
78	ASA 008760
79	ASA 008761
80	ASA 008762
81	ASA 008763
82	ASA 008764
83	ASA 008765
84	ASA 008766
85	ASA 008767
86	ASA 008768
87	ASA 008769
88	ASA 008770
89	ASA 008771
90	ASA 008772
91	ASA 008773
92	ASA 008774
93	ASA 008775
94	ASA 008776

-42-

95	ASA 008777
96	ASA 008778
97	ASA 008779
98	ASA 008780
99	ASA 008781
100	ASA 008782
101	ASA 008783
102	ASA 008784
103	ASA 008785
104	ASA 008786
105	ASA 008787
106	ASA 008788
107	ASA 008789
108	ASA 008790
109	ASA 008791
110	ASA 008792
111	ASA 008793
112	ASA 008794
113	ASA 008795
114	ASA 008796
115	ASA 008797
116	ASA 008798
117	ASA 008799
118	ASA 008800
119	ASA 008801
120	ASA 008802
121	ASA 008803
122	ASA 008804
123	ASA 008805
124	ASA 008806
125	ASA 008807
126	ASA 008808
127	ASA 008809
128	ASA 008810

-43-

129	ASA 008811
130	ASA 008812
131	ASA 008813
132	ASA 008814
133	ASA 008815
134	ASA 008816
135	ASA 008817
136	ASA 008818
137	ASA 008819
138	ASA 008820
139	ASA 008821
140	ASA 008822
141	ASA 008823
142	ASA 008824
143	ASA 008825
144	ASA 008826

While in principle any number of codes can be generated, the greatest utility is achieved by minimizing the size of the encoding unit, which will likewise minimize the chemical investment in it.

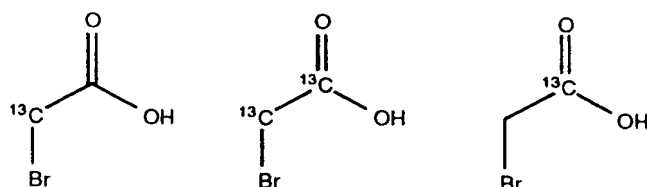
5

-44-

The disclosure of the following references is incorporated in their entirety herein by reference: Atherton, E. and Sheppard, R.C. (1989) *Solid Phase Peptide Synthesis: A Practical Approach*. IRL Press, Oxford. Bodanszky, A. and Bodanszky, M. (1984) *The Practice of Peptide Synthesis*. Springer-Verlag, Berlin.

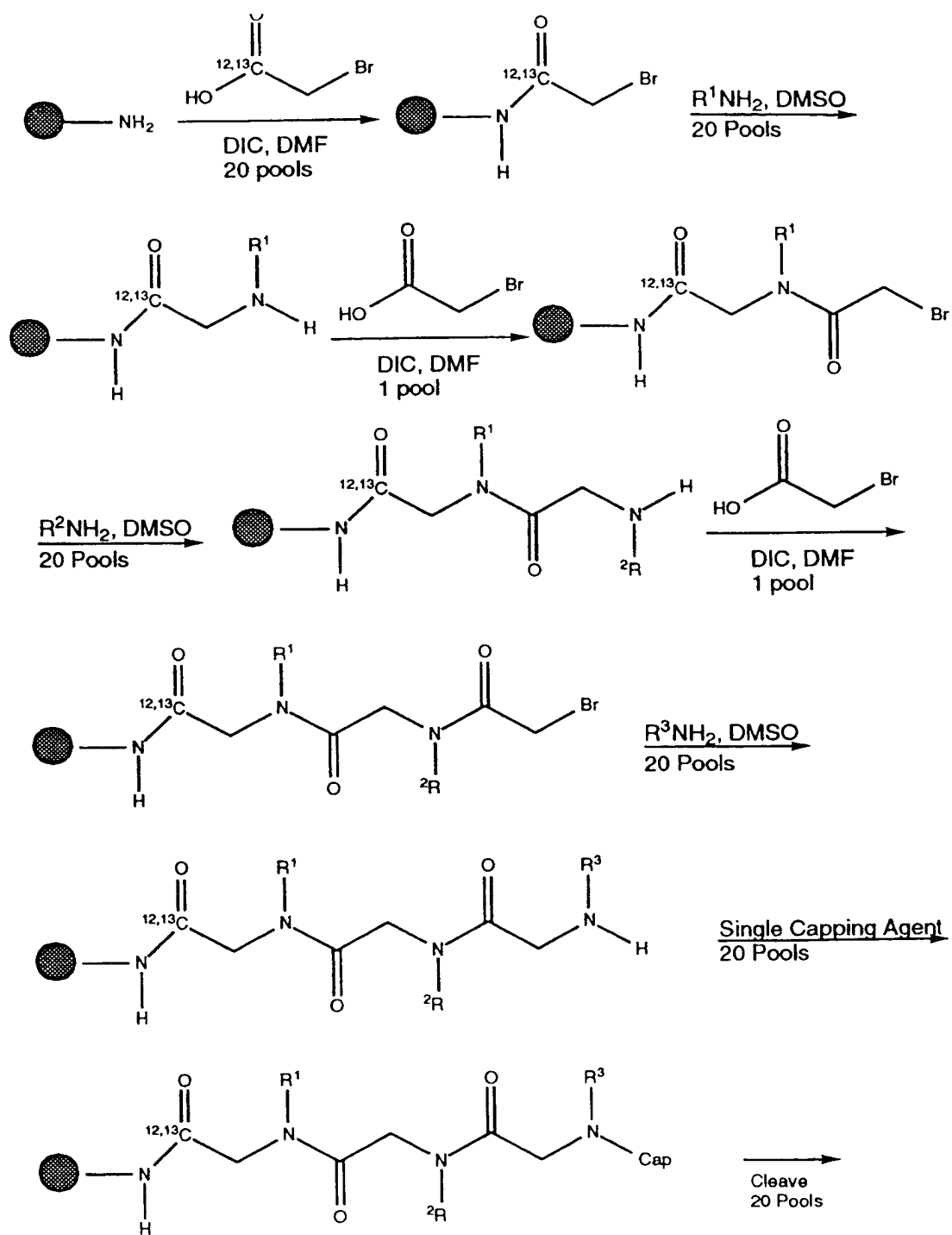
Example 2

Three commercially available (isotopically doped bromoacetic acids provide three encoding moieties

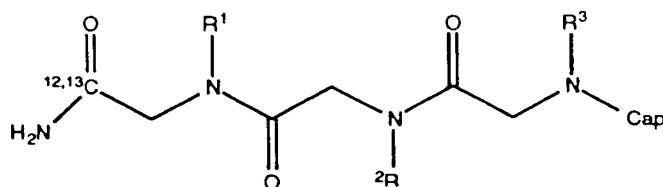


The following synthesis scheme illustrates one variant of the ratio approach. 21 Pools of bromoacetic acid, each having ratios of C^{12} to C^{13} that progress in 5% increments of from 0% to 100% of each are prepared, in this case with the isotope doping occurring at the number one carbon only.

-45-



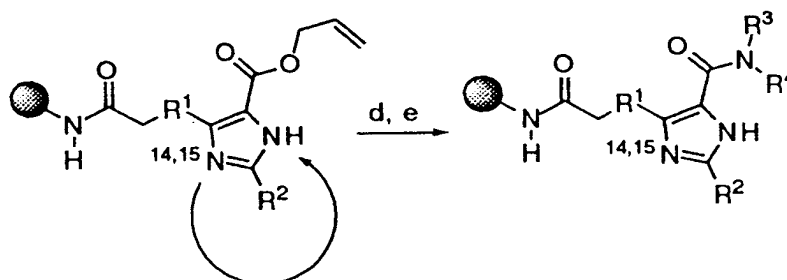
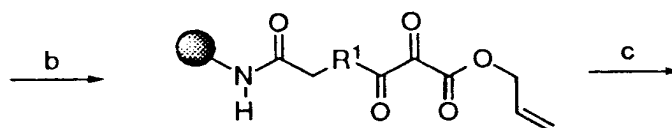
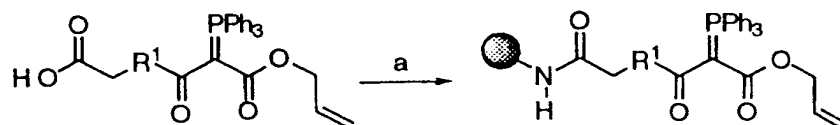
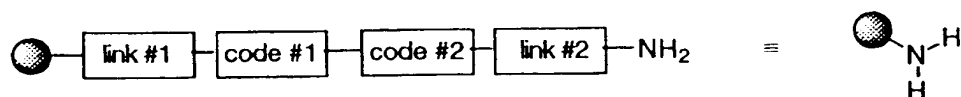
-46-



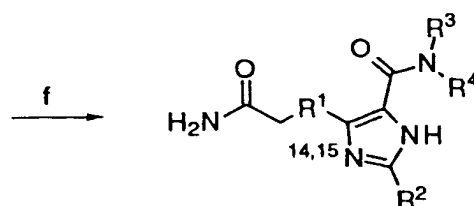
The R^1 moiety is encoded by the C^{12} to C^{13} ratio
 sample, the R^3 is encoded by the pool number from R^3 was taken,
 5 and the R^2 moiety is encoded by subtraction of the known R^1 and
 R^3 masses from the total construct mass.

10 Example 3: An Example of Isotope Encoding Using the $N^{14,15}$
Isotope Ratio in the Form of a Reagent - $N^{14,15}H_4OAc$

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14,15 N at both positions

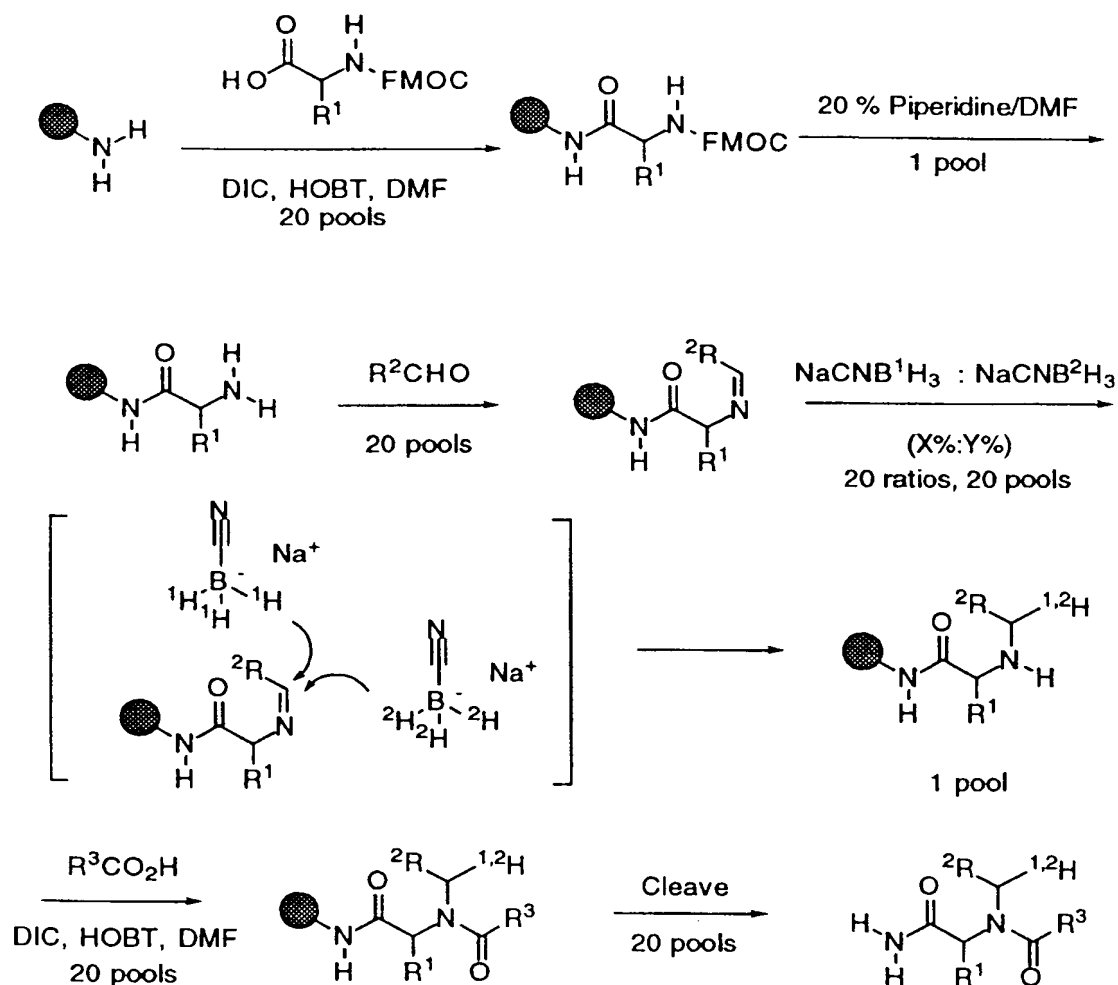


- (a) Encoded resin, 1-hydroxy-7-azabenzotriazole, diisopropylcarbodiimide, DMF; (b) trans-(±)-3-phenyl-2-(phenylsulfonyl)-oxaziridine, CHCl_3 ; (c) Aldehyde, (X%:Y%) $^{14}\text{NH}_4\text{OAc}$: $^{15}\text{NH}_4\text{OAc}$, $\text{AcOH}/\text{CHCl}_3$ (50:50); (d) (i) $\text{Pd}(\text{PPh}_3)_4$, CHCl_3 /4-methylmorpholine/ AcOH (90:5:5), (ii) sodium diethyldithiocarbamate/diisopropylethylamine/DMF (99:0.5:0.5); (e) Amine, PyBOP, diisopropylethylamine, DMF; (f) TFA/ H_2O (95:5)

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Example 4: An Example of Isotope Encoding Using the $^{1,2}\text{H}$ Isotope Ratio in the Form of a Reagent - NaCNBD_4

This example incorporates by reference the disclosure of Lebl, M. et al., *Drug Development and Research* 1994,33,146-156)

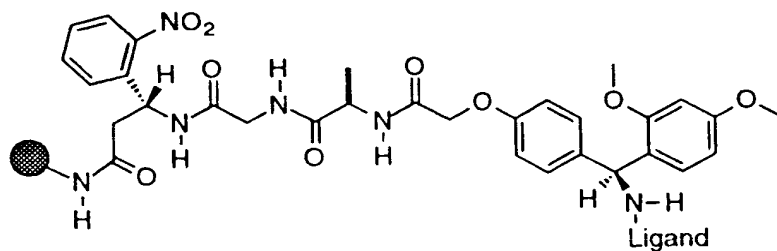
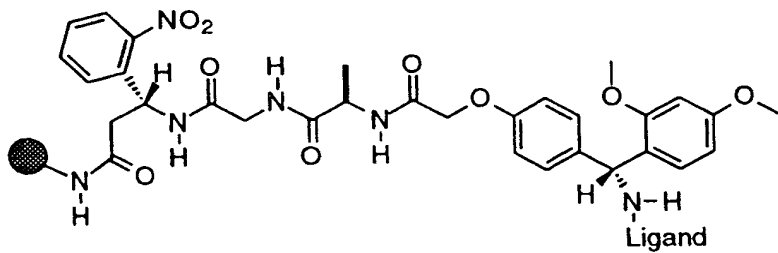
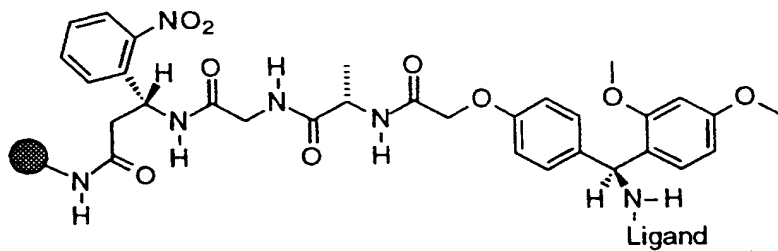
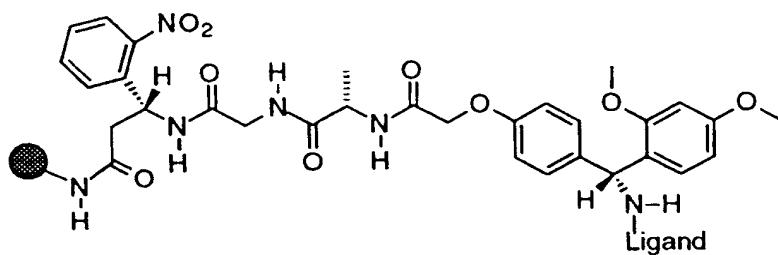


First reagent mass encodes R^1

$^{1,2}\text{H}$ ratio encodes R^2

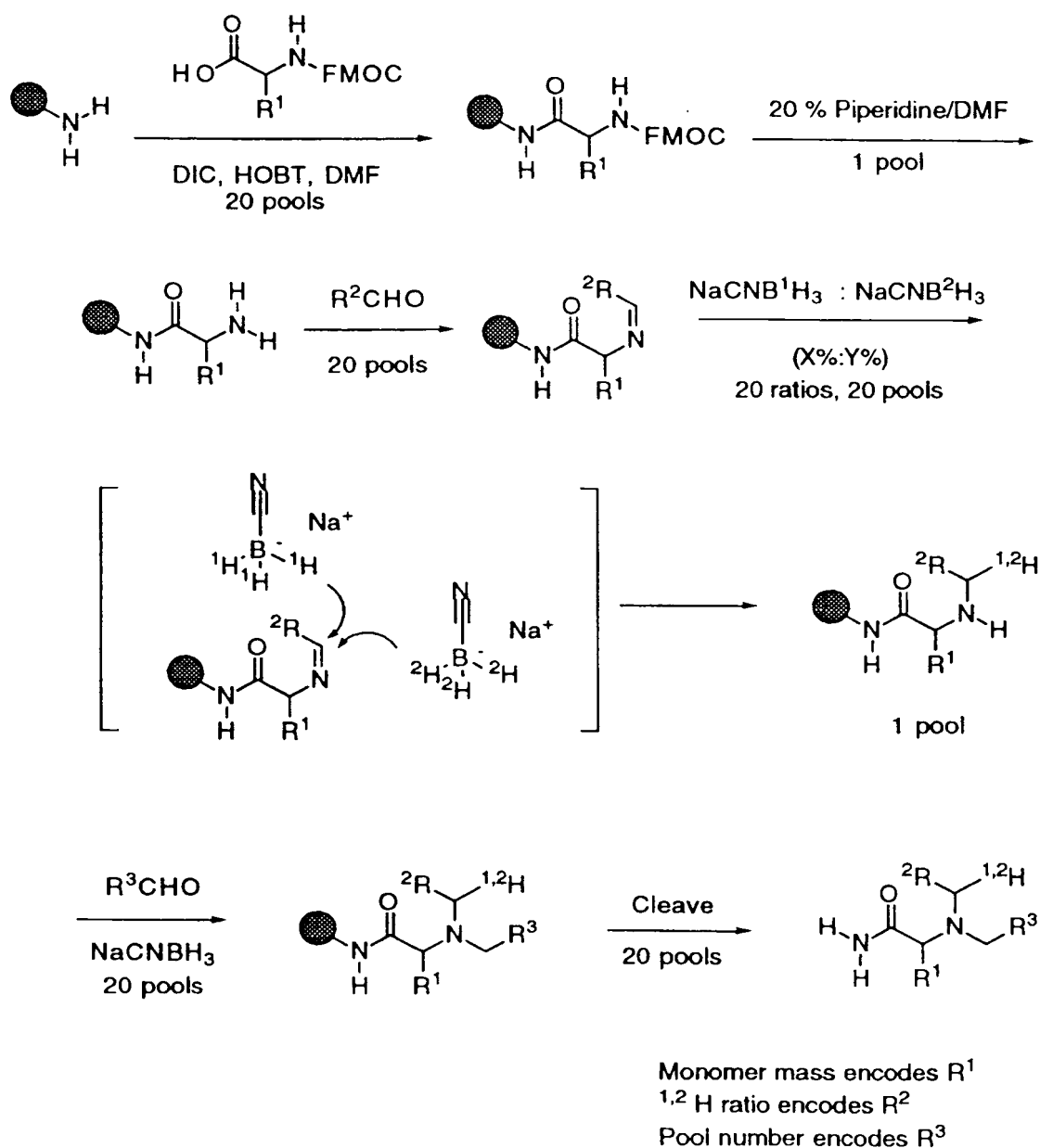
Pool number encodes R^3

-49-

Example 5: Encoding Using Four Possible Diastereomers of Link 1, Code 2, and Link 2

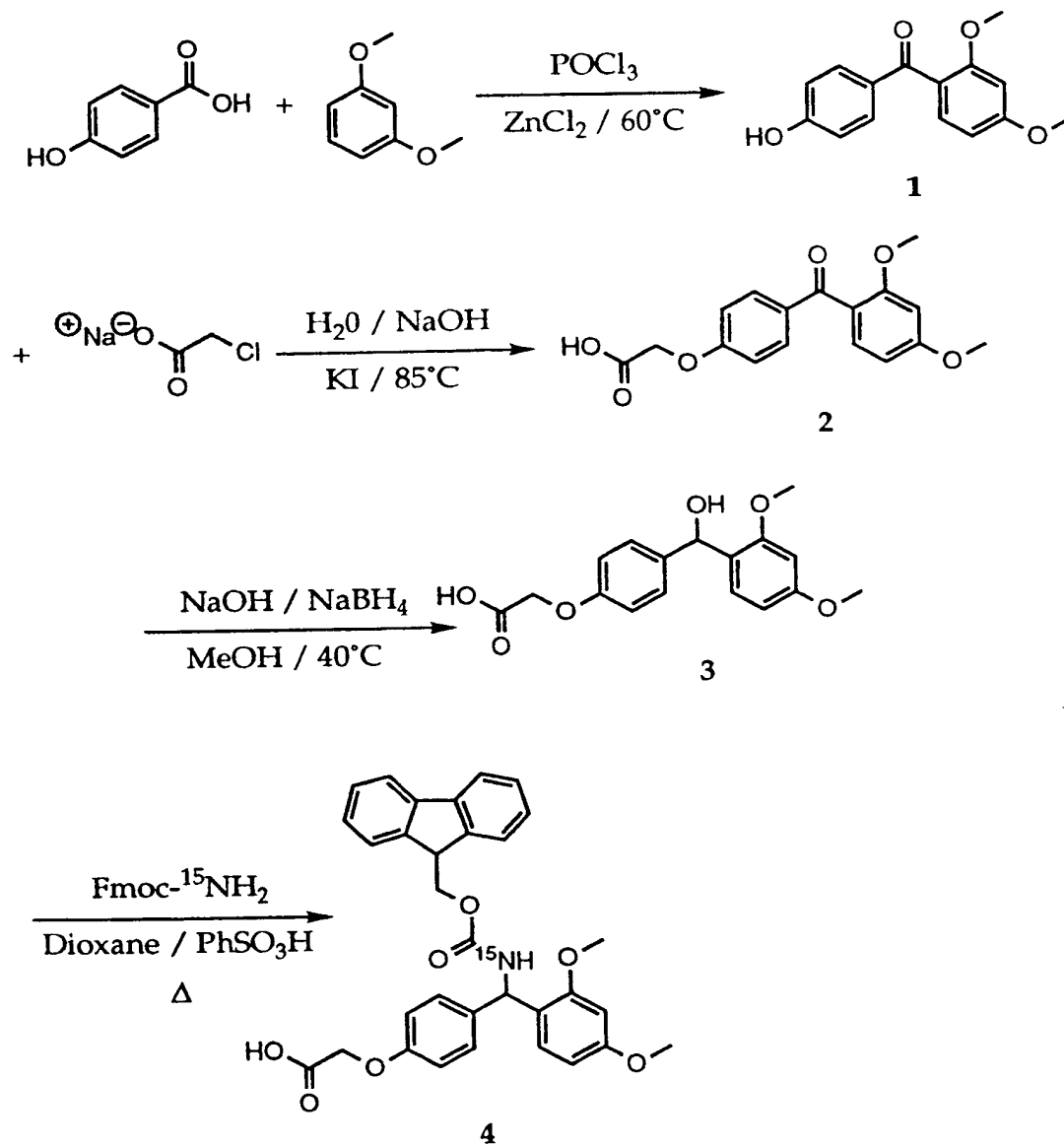
-50-

Example 6: An Example of Isotope Encoding Using the $H^{1,2}$ Isotope Ratio in the Form of a Reagent - $NaCNBD_4$

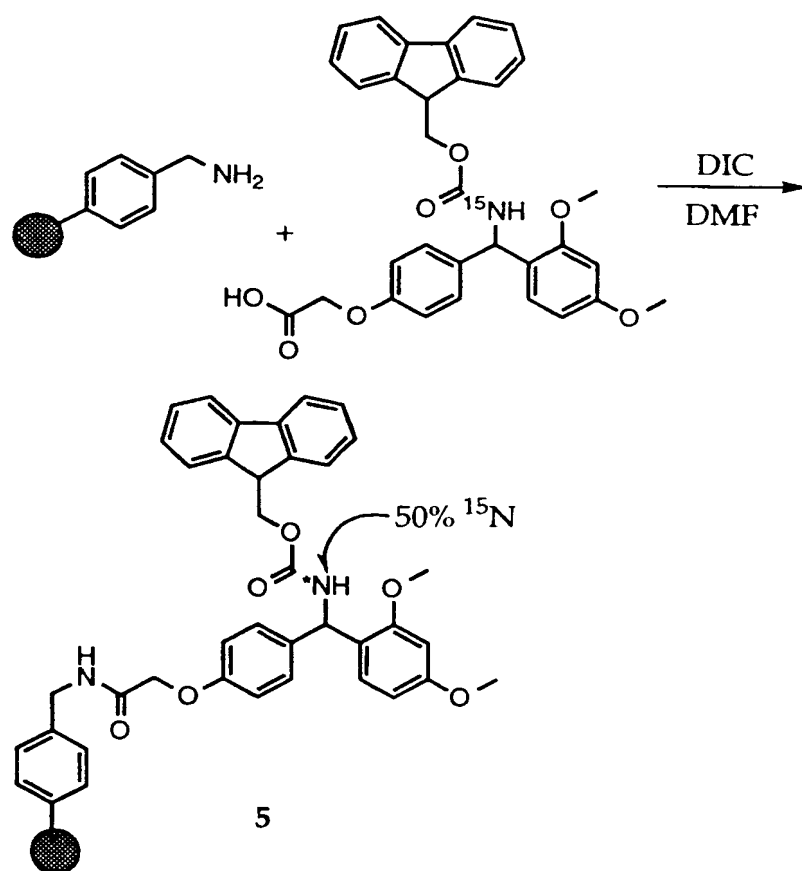
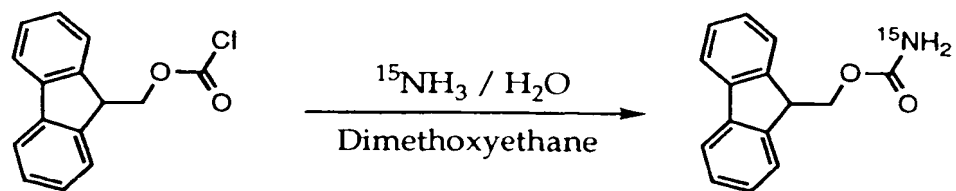


Scheme for Examples 7 through 13

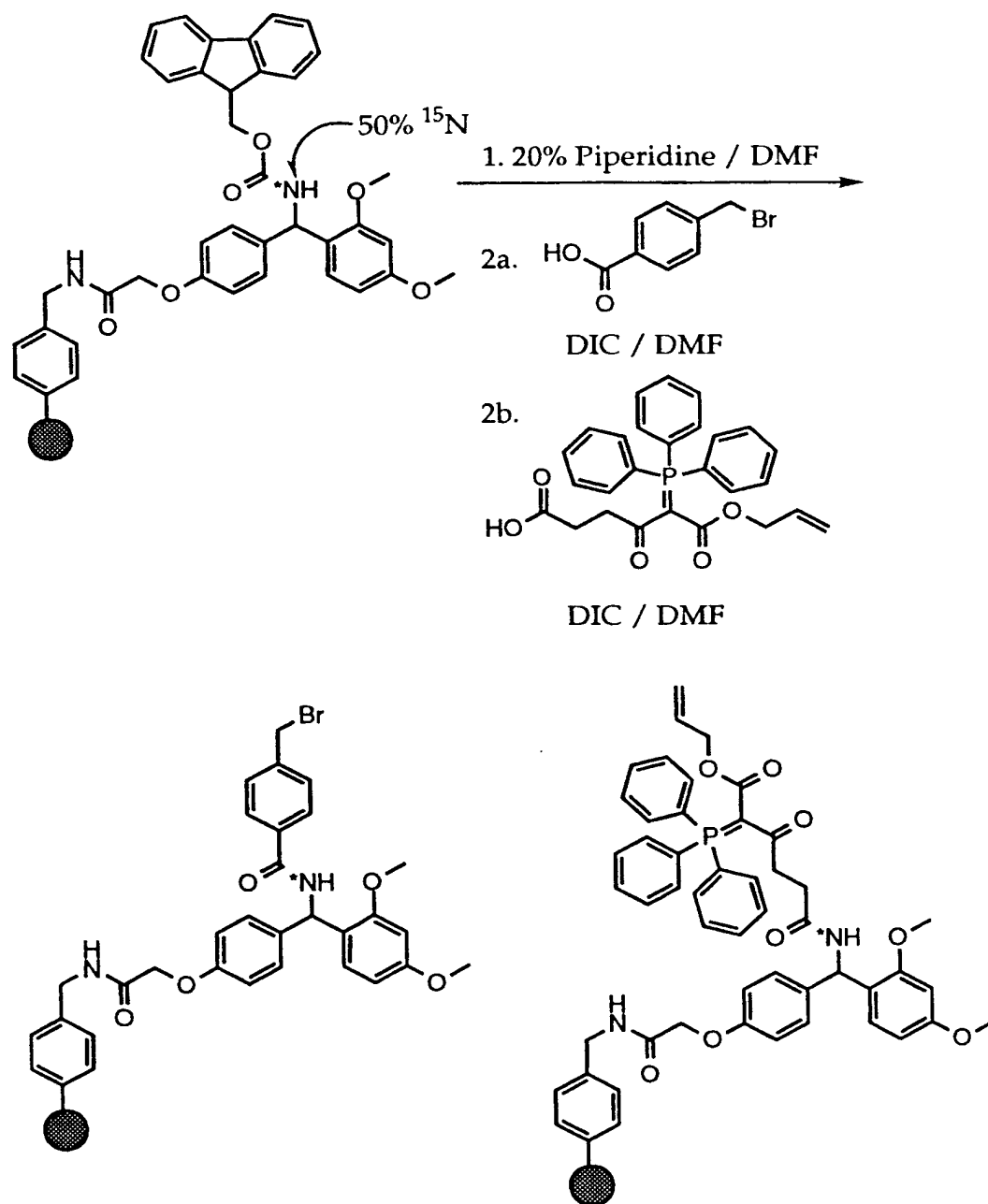
-51-



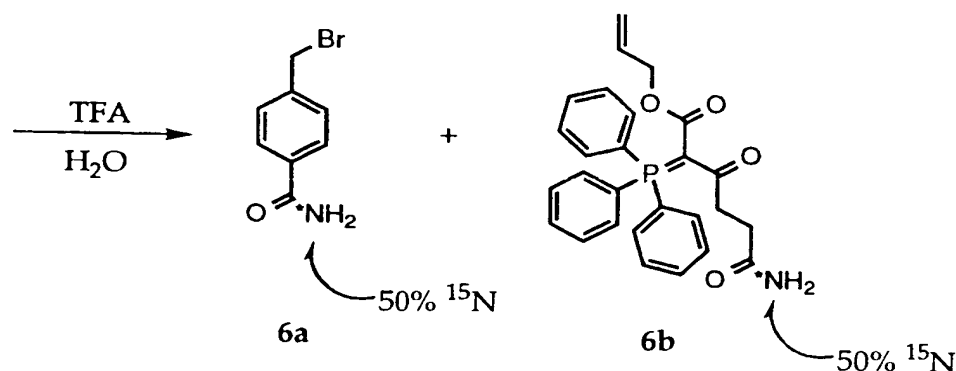
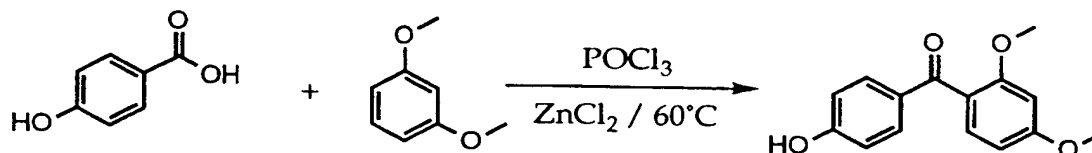
-52-



-53-



-54-

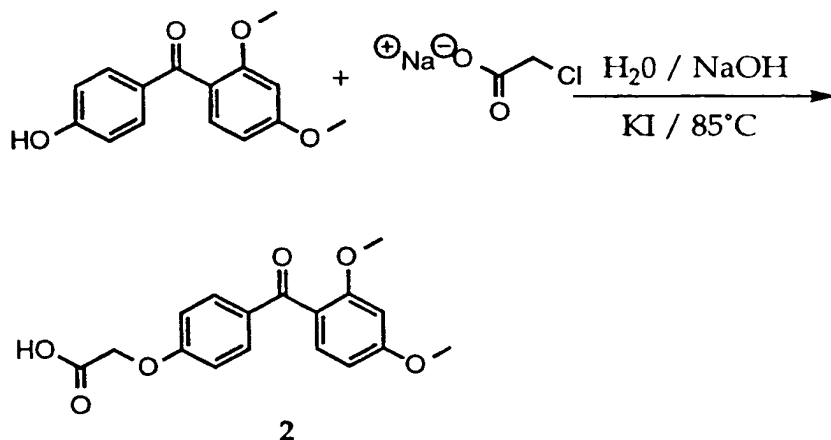
Example 7

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1 - In a 500 ml three-neck round-bottom flask, charged with a stir bar, fitted with a reflux condenser, and 60 ml addition funnel (under nitrogen), was placed 50.5 g 4-hydroxybenzoic acid, and 95 ml 1,3-dimethoxybenzene. 123.33 g zinc chloride was added and the mixture was heated to 60° with an oil bath. 126 ml phosphorus oxychloride was added drop-wise over approx. 30 min. After addition, the reaction ran for 90 min at 60°. The dark red mixture was poured over 1.5 L of ice (under heavy mixing), and to this was added 276 g sodium carbonate, in small portions (significant gas evolution was observed). 400 ml ethyl acetate was added and the aqueous was extracted with another 500 ml ethyl acetate. The organics were combined and washed twice with brine and dried over mag. sulfate, filtered, and concentrated in vacuo at 40°, and further dried under vacuum to give 142.2 g of a redish-pink solid. The solid was tritrated with 400 ml hot hexane, filtered, and dried to give 63.3 g of a pink solid. Recrystallization from 75ml hot methanol gave a first crop yield of pure 2,4-dimethoxy-4-hydroxy-benzophenone (**1**) as a light-pink solid (21.9%).

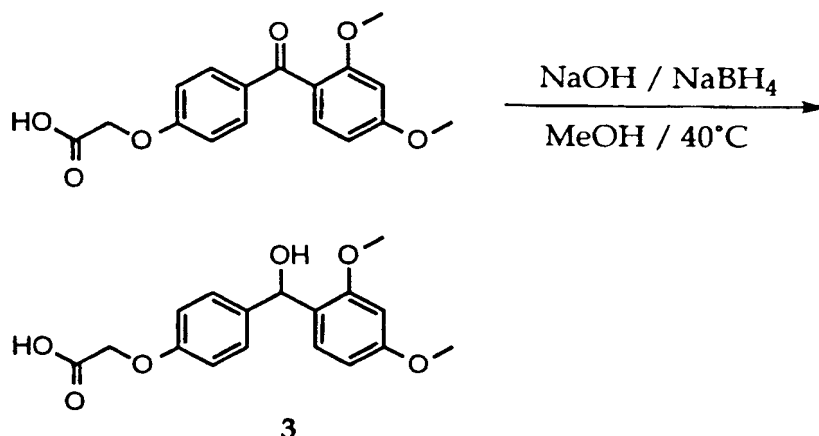
Example 8

-55-

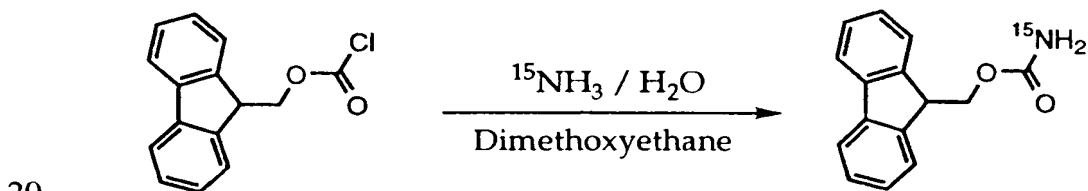


- 2 - In a 500 ml recovery flask was added 20.043 g of 2,4-dimethoxy-4-hydroxy-benzophenone, 150 ml water, 0.75 g potassium iodide, and 82.5 g sodium chloroacetate. The mixture was then heated to 85° in an oil bath. To the mixture was added 50% NaOH (w/v) in small amounts, to maintain the pH at 11-14. The reaction was followed by TLC (2/5 methanol/methylene chloride) until the reaction was complete (3 hrs). The reaction solution was transferred to a 500 ml erlenmyer flask, while still hot, and cooled to room temp. 150 ml ethyl acetate was added and the solution was acidified to pH 2 with conc. HCl. The solution mixed overnight. The large amount of white precipitate was filtered and washed 3 x 50 ml water. The off-white solid was dried under vacuum to give a first crop yield of 16.17 g pure 2 (66%)

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Example 9

- 5 **3** - In a 100 ml recovery flask was placed 1.00 g of 2, 32 ml
methanol, and 3.2 ml 1N NaOH. This clear solution was warmed
to 50° in an oil bath. To the solution was added 0.18 g sodium
borohydride in small amounts. The reaction was allowed to
10 proceed for 2.5 hrs. The reaction was then cooled to room temp.
and the solution was concentrated to a small volume. The mixture
was acidified with 2N HCl and the solution was concentrated
further to remove the methanol. The solution was extracted with
ethyl acetate. The organics were washed with brine, dried over
sodium sulfate, filtered, concentrated, and dried under vacuum to
15 give 0.98 g of a light pink foam **3** (97.5%). The product was
used immediately for the next step.

Example 10

20

- In a 200 ml recovery flask was placed 10.0 g of Fmoc-Cl, which
was dissolved in 100 ml of 80% dimethoxyethane. To this mixture
was added approx. 35 ml of 10% ¹⁵N-ammonia in water. A
25 white precipitate began to form. The pH of the mixture was
brought to 7-9. The reaction was allowed to proceed for 30 min.

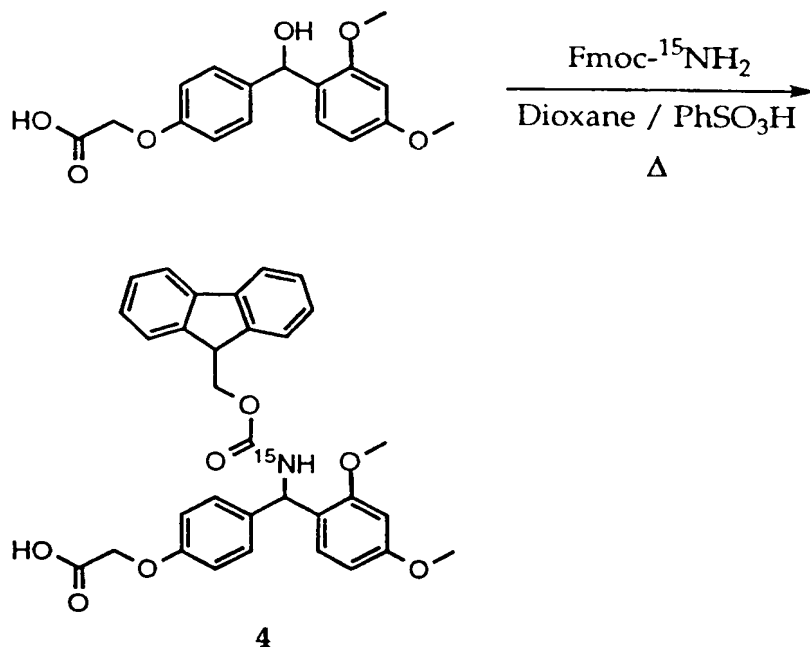
-57-

The reaction was acidified with 12N HCl and the white solid was filtered off and air dried. Further drying under vacuum gave a first crop yield of 8.072 g pure Fmoc- $^{15}\text{NH}_2$ as a white crystalline solid.

5

Example 11

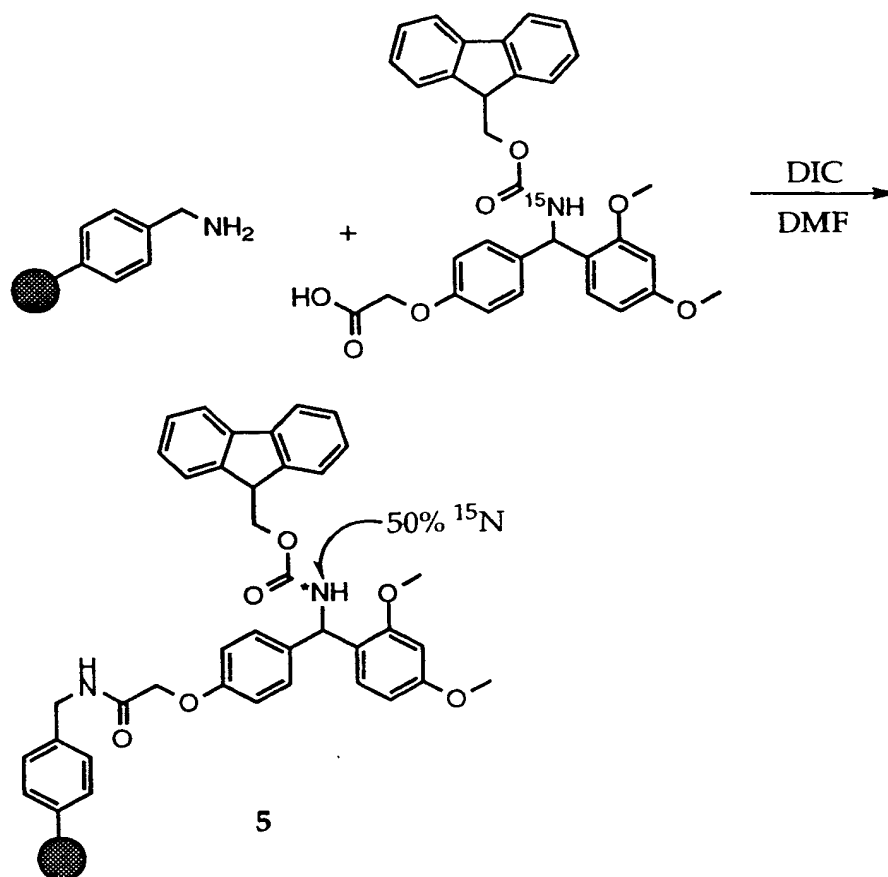
-58-



- 4 - In the 100 ml recovery flask used to dry 3 was added 20 ml
5 dioxane, 0.75 g Fmoc-¹⁵NH₂ and 0.25 g benzenesulfonic acid.
The reaction was heated to 40°C for 18 hrs. A large amount of
light gray precipitate was noted. The mixture was added to water
and the light brown solid was filtered and purified by flash col.
chromatography (100% ethyl acetate, then 98% ethyl
10 acetate/acetic acid) to give 0.37 g of pure 4 as a light-brown oil.

Example 12

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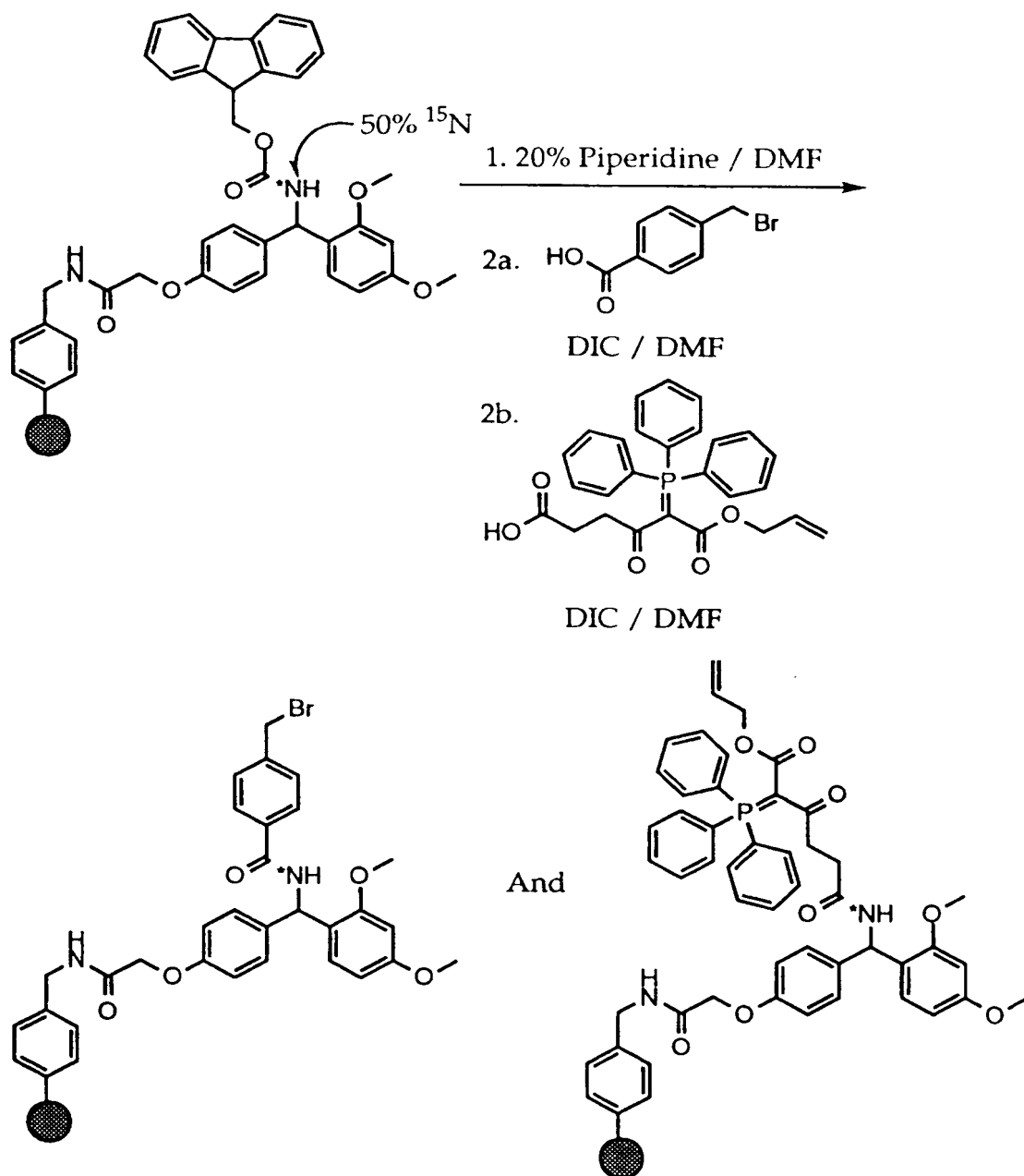


- 5 - In a 10 ml shaker tube was placed 0.50 g of aminomethylated polystyrene (0.21 mmol/g). To the resin was added a solution of 0.1845 g 4 ¹⁵N-Knorr linker and 0.184 g commercial ¹⁴N-Knorr linker in 5 ml DMF, and 0.164 ml diisopropylcarbodiimide. The coupling ran on a shaker for 5 hrs. The coupling solution was filtered off, and the resin was washed with DMF and CH₂Cl₂, and dried under vacuum (ninhydrin negative) to provide 5.

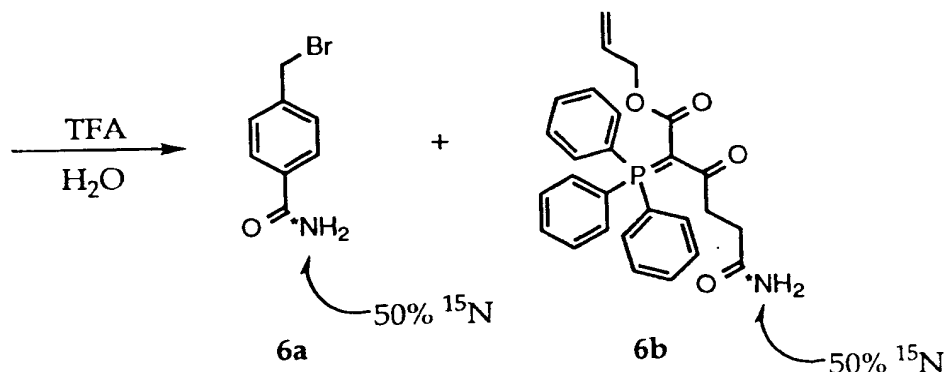
10

Example 13

-60-



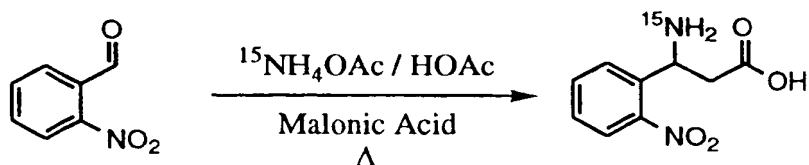
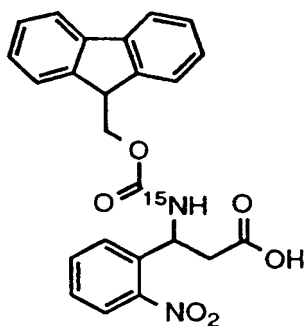
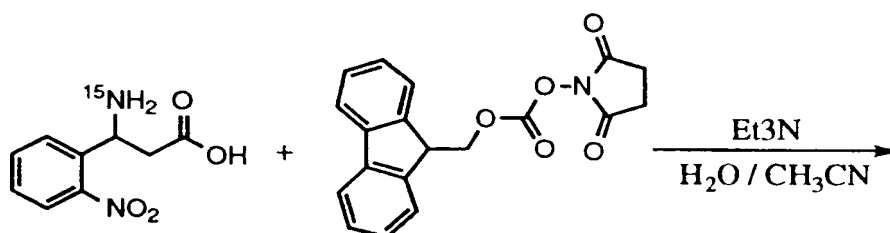
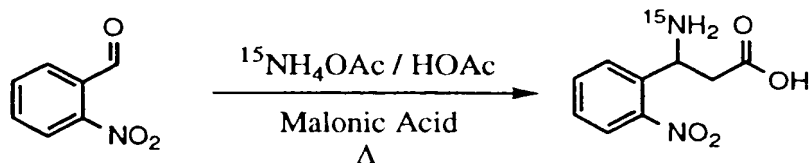
-61-



- 6a & 6b** - In two 5 ml shaker tubes, each with approx 10 mg of resin 5, 2 x 4 ml of 20% piperidine/DMF was added to both.
- 5 Both were then washed with DMF (ninhydrin pos). To the first shaker was added 0.0246 g of 4-bromomethyl benzoic acid, 0.05 ml DIC, in 1.0 ml DMF. To the second was added 0.0168 of the ylide-acid, 0.05 ml DIC, in 1.0 ml DMF. Each reaction ran on a shaker overnight. The resins were washed with DMF, methylene
- 10 chloride, and dried under vacuum (ninhydrin negative). To the dry resins was added 1.0 ml of 95% TFA/water and the cleavage ran for 1 hr. Filtered the solutions and concentrated to give **6a** and **6b**. ESI-MS showed an aprox. 46% ^{15}N ratio.

15 Example 14-Preparation of Mass-labeled photocleavable linkers

-62-

**1**

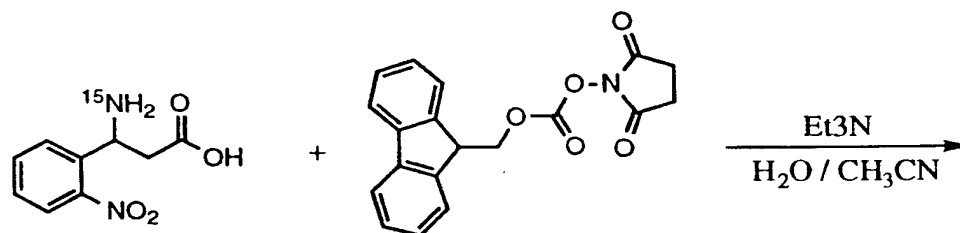
5 Example 15-Ratio Codes

1 - A solution of 1.511 g 2-Nitrobenzaldehyde (10.0 mmol), 1.563 g malonic acid (15.0 mmol), 2.00g ^{15}N (98%)-ammonium acetate (25.6 mmol), in 5.0 ml acetic acid (99.999%) was heated to 100° with an oil bath. As noted in the Oelschläger

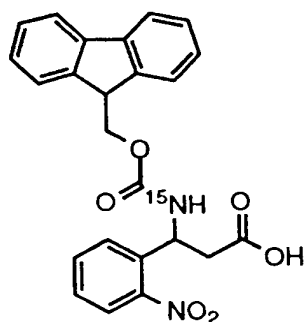
-63-

experimental, during heating there was a temporary, almost colorless precipitate (ammonium salt of the benzyliden compound). After 5 hours of heating, 8.0 ml of 25% HCl was added and heating continued for another 5 hours. To this mixture
 5 was added 12.0 ml of water and the reaction was cooled to room temperature. A light brown precipitate was filtered off and the filtrate was evaporated to almost dryness. The solution was then briefly boiled with activated carbon and filtered. The filtrate was made basic with the addition of concentrated ammonium
 10 hydroxide. 1 precipitated as a yellowish solid. The solid was then washed twice with water, once with 50% methanol in water, once with 1:1 methanol-ether, and three times with ether. The solid was then dried under vacuum to yield 0.8 g pure product 1 (38%).

15



1



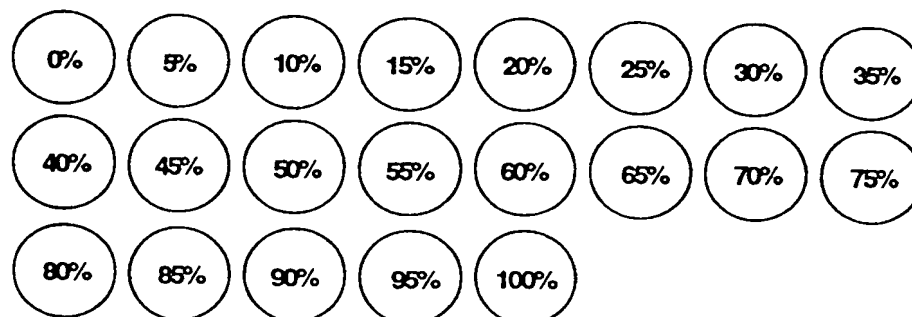
2

0.470 g (2.22 mmol) of 1 was suspended in 5.0 ml water, and subsequently dissolved by the addition of 0.299 (2.13 mmol) ml triethylamine. To this solution was added 0.757 g Fmoc-OSU
 20 (2.25 mmol) in 5.0 ml acetonitrile. The reaction then ran for 30

-64-

min. (pH \approx 10). The slurry was evaporated and diluted with 5.0 ml water and 10.0 ml ethyl acetate. The pH was adjusted to 2 with 12N HCl. A white solid precipitated. Filtered and extracted with ethyl acetate. The organics were washed with 2N HCl, H₂O, and brine, dried over mag. sulfate, filtered and evaporated to give a white solid which upon trituration with ethyl acetate and hexane gave 0.36 g pure **2** (0.37%).

Well array:



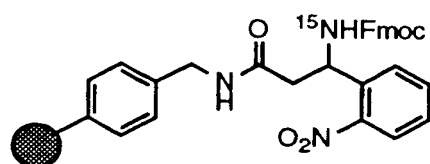
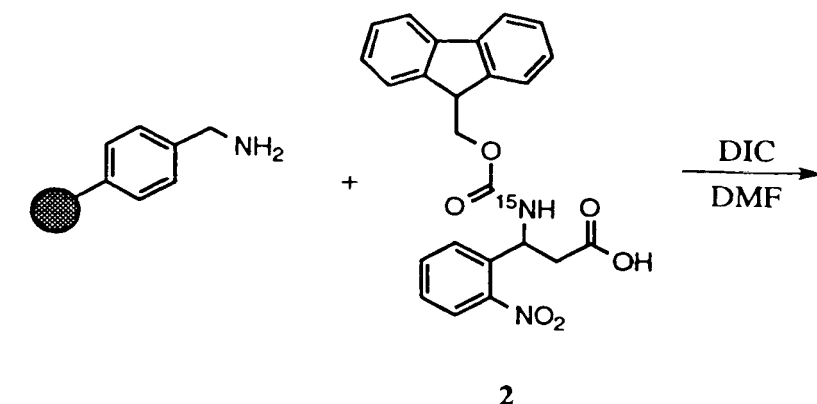
Percentage/ratio solutions of ¹⁴N/¹⁵N-photolinker were generated in the following manner (from 10.5 ml stock solutions DMF of each photolinker).

0% = 20 x 0.050 ml of 10.5 ml ¹⁴N-photolinker, and 0 x 0.050 ml of 10.5 ml ¹⁵N-photo
 5% = 19 x 0.050 ml of 10.5 ml ¹⁴N-photolinker, and 1 x 0.050 ml of 10.5 ml ¹⁵N-photo
 10% = 18 x 0.050 ml of 10.5 ml ¹⁴N-photolinker, and 2 x 0.050 ml of 10.5 ml ¹⁵N-photo
 15% = 17 x 0.050 ml of 10.5 ml ¹⁴N-photolinker, and 3 x 0.050 ml of 10.5 ml ¹⁵N-photo
 20% = 16 x 0.050 ml of 10.5 ml ¹⁴N-photolinker, and 4 x 0.050 ml of 10.5 ml ¹⁵N-photo
 25% = 15 x 0.050 ml of 10.5 ml ¹⁴N-photolinker, and 5 x 0.050 ml of 10.5 ml ¹⁵N-photo
 30% = 14 x 0.050 ml of 10.5 ml ¹⁴N-photolinker, and 6 x 0.050 ml of 10.5 ml ¹⁵N-photo

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- 35% = 13 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 7 x 0.050 ml of 10.5 ml ^{15}N -photo
- 40% = 12 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 8 x 0.050 ml of 10.5 ml ^{15}N -photo
- 5 45% = 11 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 9 x 0.050 ml of 10.5 ml ^{15}N -photo
- 50% = 10 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 10 x 0.050 ml of 10.5 ml ^{15}N -photo
- 55% = 9 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 11 x 0.050 ml of 10.5 ml ^{15}N -photo
- 10 60% = 8 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 12 x 0.050 ml of 10.5 ml ^{15}N -photo
- 65% = 7 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 13 x 0.050 ml of 10.5 ml ^{15}N -photo
- 15 70% = 6 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 14 x 0.050 ml of 10.5 ml ^{15}N -photo
- 75% = 5 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 15 x 0.050 ml of 10.5 ml ^{15}N -photo
- 80% = 4 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 16 x 0.050 ml of 10.5 ml ^{15}N -photo
- 20 85% = 3 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 17 x 0.050 ml of 10.5 ml ^{15}N -photo
- 90% = 2 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 18 x 0.050 ml of 10.5 ml ^{15}N -photo
- 25 95% = 1 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 19 x 0.050 ml of 10.5 ml ^{15}N -photo
- 100% = 0 x 0.050 ml of 10.5 ml ^{14}N -photolinker, and 20 x 0.050 ml of 10.5 ml ^{15}N -photo

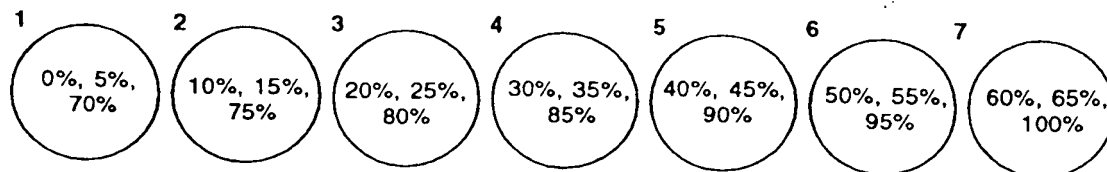
-66-



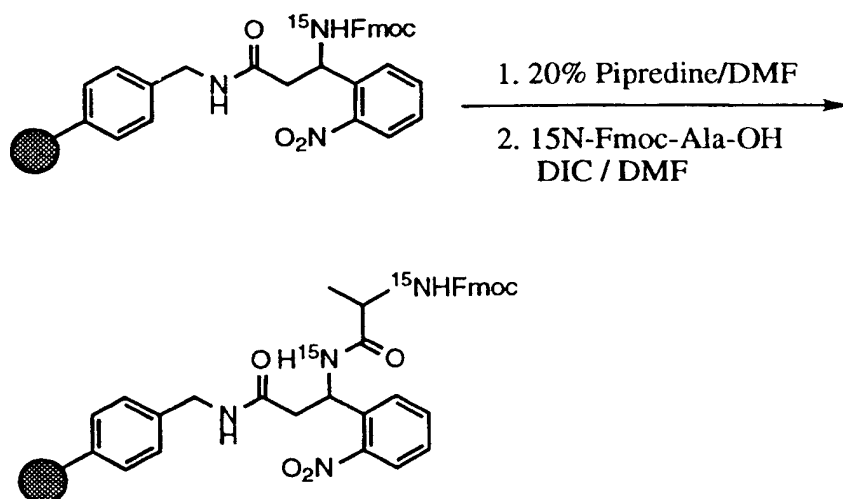
- Starting with 6.3 g of aminomethyl polystyrene (0.21 mmol/g), divided into 21 equal lots of 0.300 grams each, were added the
- 5 1.0 ml solutions of varying ratios of ^{15}N -2 and ^{14}N -2, in 5% increments (0%, 5%, 10%, to 100% ^{15}N -2 photolinker) (see CDW fig. 1). These solutions were generated by dissolving 0.4988 g ^{14}N -2 photolinker and 0.500 g ^{15}N -2 photolinker, and using 0.050 ml aliquots of each (20:0, 19:1, 18:2, to 0:20). To the
- 10 mixture was then added 0.169 ml diisopropylcarbodiimide. The samples were then mixed and the coupling ran overnight (approx. 16 hours). The resins were washed 8 times with 1.5 ml DMF, and then with copious amounts of methanol.
- 15 Approx. 0.1 g of each lot was combined into 7 lots consisting of the following ratios per lot (see CDW fig. 2). These 7 resin lots were used in the Tertiary Amine Library Study.

Well array:

20



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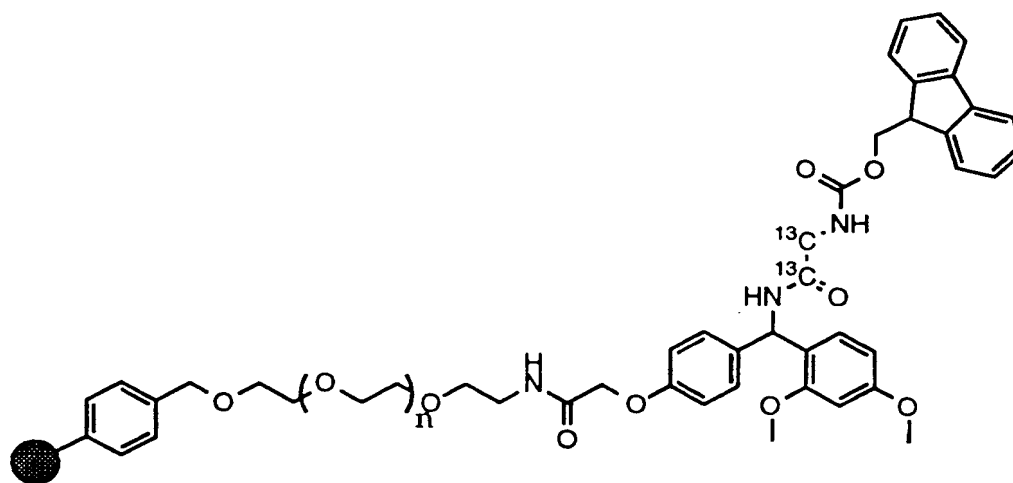


- 5 Approx. 0.1 g of each resin lot ratio was coupled with 0.50 ml of a 10.5 ml, 0.55 g 15N-Fmoc-Alanine-OH in DMF solution, followed by the addition of 0.169 ml DIC. The coupling then ran overnight. The resins were washed with copious amounts of methanol and dried under vacuum (ninhydrin negative).
- 10 A small amount of each resin lot, approx 0.01 g, was photo-cleaved for 4hrs in 0.200 ml 3:1 water/THF. MS was subsequently performed.

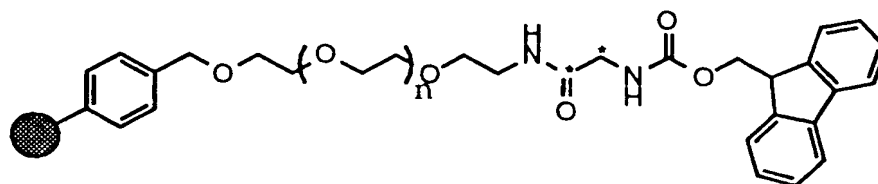
15 Example 16-Preparation of mass-based codes distinguishable by NMR

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Novabiochem TGR resin coupled with $^{13}\text{C}_2$ -Fmoc-gly-OH. Both the carbonyl carbon and methylene carbon of glycine were evident by ^{13}C NMR of the resin in CDCl_3 . Additionally, these carbons could be integrated against the standard PEG signal that would remain constant throughout & the Ratios of ^{13}C linker/ligand could be varied and subsequently determined (ligand resolution) by this non-destructive method.

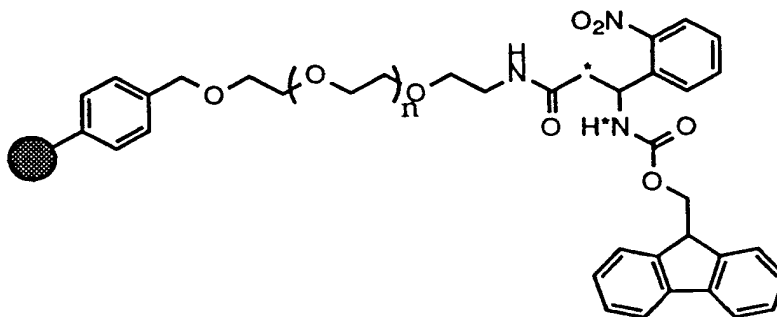


Novabiochem TG resin coupled with $^{13}\text{C}_2$ -Fmoc-gly-OH



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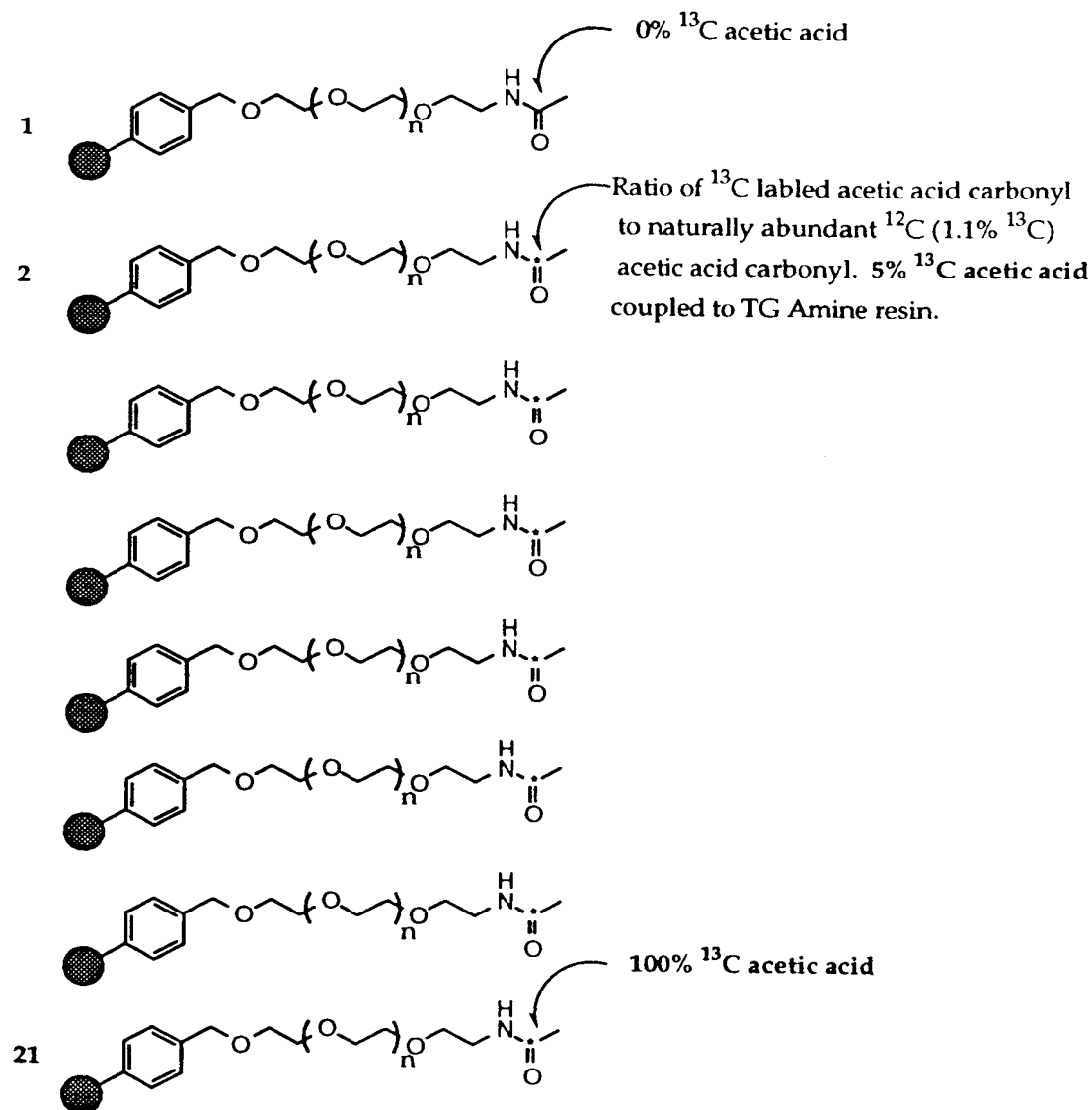
Novabiochem TG resin coupled with ^{13}C , ^{15}N -Photolinker. This linker could have a hard ratio ^{13}C to ^{13}C tag, as well as a cleavable ^{15}N tag.



Example 17-NMR Ratio Coding Approach

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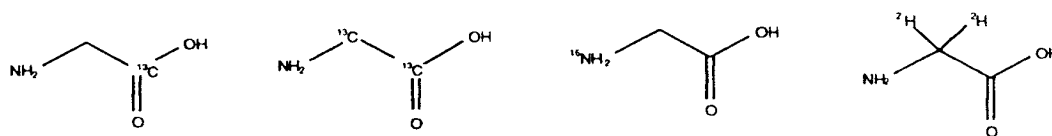
^{13}C NMR quantitation study on a ratio of ^{13}C labeled acetic acid to non-labeled acetic acid bound to Tenta-Gel- NH_2 resin. The ^{13}C acetic acid carbonyl can be integrated against naturally occurring ^{13}C in the PEG portion of the TG- NH_2 resin. A differentiation of 5% increments is observed.



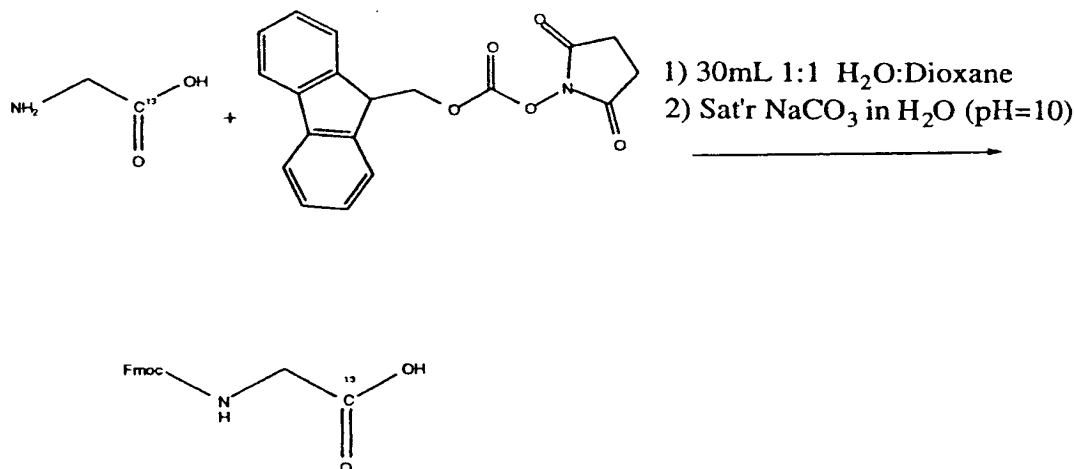
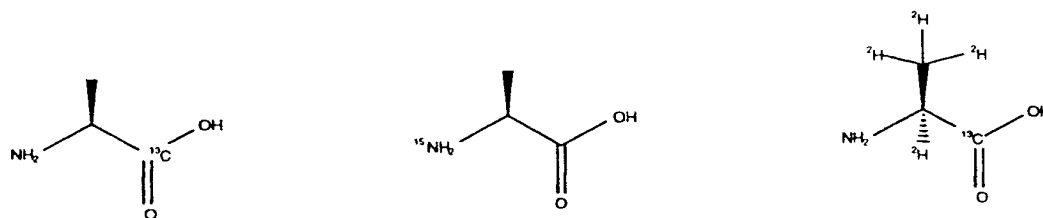
Example 18-Preparation of Fmoc-protected isotopically doped code blocks

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Isotope Labeled Glycine from Cambridge Isotope Laboratories



Isotope Labeled Alanine from Cambridge Isotope Laboratories

*N-Fmoc-L-glycine*

5

1 g of each isotope labeled glycine (13.3 mmol) were dissolved in 15 mL of purified water and 1.4 g of sodium carbonate (see Figure GP1). 15 mL of 1,4-Dioxane was added slowly to each and stirred. Over the period of two hours, 4.5 g of Fmoc-OSu (9-Fluorenylmethyl-N-hydroxysuccinimide) was added to each

10

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(see Figure GP3 for scheme). Additional saturated sodium carbonate in water was added to maintain a pH=10. Solutions were allowed to stir overnight. 6M HCl was slowly added to the solution until the pH was approximately 2. Extraction of the compound was completed using 50 mL of ethyl acetate, washing four times with 50 mL of acidified/purified water. Aqueous portions were washed with 50 mL of ethyl acetate. Both portions of ethyl acetate were combined. Magnesium sulfate was added to remove any excess water. Samples were filtered and concentrated using a RotoVap until the final volume was approximately one-third the original volume. Crystallization was completed using Hexane. Hexane was decanted and compound allowed to dry.

15 *N-Fmoc-L-alanine*

1 g of each isotope labeled alanine (11.2 mmol) were dissolved in 15 mL of purified water and 1.2 g of sodium carbonate (see Figure GP2). 15 mL of 1,4-Dioxane was added slowly to each and stirred. Over the period of two and a half hours, 3.8 g of Fmoc-OSu (9-Fluorenylmethyl-N-hydroxysuccinimide) was added to each (see Figure GP3 for scheme). Additional saturated sodium carbonate in water was added to maintain a pH=10. Solutions were allowed to stir overnight. 6M HCl was slowly added to the solution until the pH was approximately 2. Extraction of the compound was completed using two times 150 mL of ethyl acetate, washing four times with 300 mL of acidified/purified water. Magnesium sulfate was added to remove any excess water. Samples were filtered and concentrated using a RotoVap until the final volume was approximately one-third the original volume. Crystallization was completed using Hexane. Hexane was decanted and compound allowed to dry.

The disclosure of the following references is incorporated in their entirety herein by reference: Atherton, E. and Sheppard, R.C. (1989) *Solid Phase Peptide Synthesis: A Practical Approach*. IRL Press, Oxford. Bodanszky, A. and Bodanszky, M. (1984) *The Practice of Peptide Synthesis*. Springer-Verlag, Berlin.

40

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Example 18-Synthesis of Isotopically Labeled Peptides for Mass Spectral Analysis, Showing Their Use in Four Encoding Approaches

5 Fmoc-protected alanine, serine and lysine were purchased from Bachem Bioscience. Fmoc-glycine was purchased from Novabiochem. Isotopically labeled amino acids were purchased from Cambridge Isotope Laboratories and were Fmoc protected in house (GP). Fmoc Knorr linker was purchased from
10 Novabiochem. Fmoc-3-(2-nitrophenyl)-3-aminopropionic acid (a photolabile linkage agent) was purchased from Universal Organics. Aminomethyl polystyrene resin (0.81 mmol/gram) was purchased from Advanced ChemTech.

Linkers were attached to solid support manually in reaction
15 shakers (CM). Resin was split to 85 reaction vessels on an Advanced ChemTech ACT496 MBS. Resin was deprotected with 25% piperidine/dimethylformamide twice for 10 minutes, then washed thoroughly with dimethylformamide. The first amino acids were coupled as 0.142 M solutions in N-methyl-pyrrolidone,
20 with PyBOP/Diisopropylethylamine in situ activation for 1.5 hours at room temperature. Boc deprotection was performed with 25 % trifluoroacetic acid in dichloromethane for 30 minutes. Acetylations were performed with 33 % acetic anhydride in dimethyl formamide for 30 minutes. All couplings were verified
25 by ninhydrin color tests. Cleavage from the knorr linker was accomplished in 90% trifluoroacetic acid, 3% each water, phenol and thioanisole for 1.5 hours. Samples were dried under a nitrogen stream, then dissolved in water and lyophilized twice. Photocleavage was performed in 2:1 water:tetrahydrofuran under
30 uv lamp for 12 hours. Samples were lyophilized once.

Single Peak Positional Code

35 This array was designed to produce 20 samples with singlet, unique molecular weights for positive identification by mass spectroscopy. The samples were assembled in duplicate, one set of twenty on a chemically cleavable solid support, and the second set of twenty on a photocleavable support. By using a combination of one or two amino acids, a "coding region" was
40 generated before addition of the synthetic ligand (in this case

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Acetyl-lysine). The twenty codes were constructed from the following amino acids :

- Glycine
- 5 Glycine (15N)
- Glycine (2,2-D2)
- Alanine
- Alanine (15N)
- Alanine (1-13C)
- 10 Serine

After the coding region had been assembled, lysine was coupled as a test ligand as well as to generate a free amino side chain for ease of observation by mass spectroscopy. The α -amino group of the lysine was then acetylated.

The codes and ligand were assembled on polystyrene resin functionalized with either Fmoc-Knorr or Fmoc-3-(2-nitrophenyl)-3-aminopropionic acid linkers.

20

The array for the single peak positional code is shown below :

Figure 1

	Position 1	Position 2	Position 3	Total MW
1	Ac- Lys-	X	X	187
2	Ac- Lys-	X	-Gly0-	244
3	Ac- Lys-	X	-Gly1-	245
4	Ac- Lys-	X	-Gly2-	246
5	Ac- Lys-	X	-Ala0-	258
6	Ac- Lys-	X	-Ala1-	259
7	Ac- Lys-	X	-Ala4-	262

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8	Ac-	X	-Ser-	274
	Lys-			
9	Ac-	-Gly0-	-Gly0-	301
	Lys-			
1	Ac-	-Gly0-	-Gly1-	302
0	Lys-			
1	Ac-	-Gly1-	-Gly1-	303
1	Lys-			
1	Ac-	-Gly2-	-Gly1-	304
2	Lys-			
1	Ac-	-Gly2-	-Gly2-	305
3	Lys-			
1	Ac-	-Ala0-	-Gly0-	315
4	Lys-			
1	Ac-	-Ala1-	-Gly0-	316
5	Lys-			
1	Ac-	-Ala0-	-Gly2-	317
6	Lys-			
1	Ac-	-Ala1-	-Gly2-	318
7	Lys-			
1	Ac-	-Ala4-	-Gly2-	321
8	Lys-			
1	Ac-	-Ser-	-Gly0-	331
9	Lys-			
2	Ac-	-Ser-	-Gly1-	332
0	Lys-			

Double Peak Positional Code

- 5 This array was designed to produce 20 samples with doublet peaks by mass spectroscopy; one peak arising from the ligand attached to a unique code, with a second peak resulting from a simultaneously assembled reference peak (ligand plus a fixed residue). As described in the SPPC above, by using a
- 10 combination of one and two amino acids, a "coding region" was generated before addition of the synthetic ligand (in this case Acetyl-lysine). The twenty codes were also constructed from the following amino acids :

Glycine

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Glycine (15N)
 Glycine (2,2-D2)
 Alanine
 Alanine (15N)
 5 Alanine (1-13C)
 Serine

The codes and ligand were assembled on polystyrene resin
 functionalized with Fmoc-Knorr of Fmoc-3-(2-nitrophenyl)-3-
 10 aminopropionic acid linkers.

The array for the double peak positional code is shown below :

15 Figure 2

	Position 1	Position 2	Position 3	Mwt
1	Ac- Lys-	X	X	187
2	Ac- Lys-	X	-Gly0-	244
3	Ac- Lys-	X	-Gly1-	245
4	Ac- Lys-	X	-Gly2-	246
5	Ac- Lys-	X	-Ala0-	258
6	Ac- Lys-	X	-Ala1-	259
7	Ac- Lys-	X	-Ala4-	262
8	Ac- Lys-	X	-Ser-	274
9	Ac- Lys-	-Gly0-	-Gly0-	301
1	Ac- Lys-	-Gly0-	-Gly1-	302
0	Lys-			
1	Ac- Lys-	-Gly1-	-Gly1-	303
1	Lys-			

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1	Ac-	-Gly2-	-Gly1-	304
2	Lys-			
1	Ac-	-Gly2-	-Gly2-	305
3	Lys-			
1	Ac-	-Ala0-	-Gly0-	315
4	Lys-			
1	Ac-	-Ala1-	-Gly0-	316
5	Lys-			
1	Ac-	-Ala0-	-Gly2-	317
6	Lys-			
1	Ac-	-Ala1-	-Gly2-	318
7	Lys-			
1	Ac-	-Ala4-	-Gly2-	321
8	Lys-			
1	Ac-	-Ser-	-Gly0-	331
9	Lys-			
2	Ac-	-Ser-	-Gly1-	332
0	Lys-			

In each of the samples produced in this experiment, the peptide molecular weight shown above will appear superimposed with the reference peak generated by Ac-Lys-Gly-NH₂. This is to demonstrate the ability to identify both the first pooling lot and the molecular ion generated by the unknown ligand.

To produce samples displaying the coded double peak, an orthogonal protection scheme was used. The first Fmoc-protected amino acid of the coding region was coupled simultaneously with an equimolar amount of Boc-glycine. The coding region was assembled using Fmoc chemistry, then the Boc protecting group was removed with trifluoroacetic acid. After a final removal of the code Fmoc protecting group with piperidine (which concomittently neutralized TFA salts on the exposed amine of glycine), the ligand (acetyl lysine) was coupled. Upon photocleavage, two compounds were liberated in equimolar amounts : the ligand linked to a reference amino acid (glycine) and the ligand linked to a unique code. The mass difference between the two peaks indicates the identity of the code, and hence the identity of the first monomer of the ligand coupled.

Mass Spectral Barcode

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This array will produce twenty five samples, each displaying a unique molecular ion or peak pattern by mass spectroscopy. By using only combinations of isotopically labeled glycine at only two positions, twenty five unique "barcodes" are produced. In this manner the first pooled lot of any single bead derived from a split-combine library can be readily identified.

In this experiment Fmoc-protected amino acids were coupled either as discretes or equimolar mixtures of two or three amino acids.

The array for the mass spectral barcode is shown below :

Figure 3

	Position 1	Position 2	Position 3	Peak Pattern
1	Ac-Lys-	-G0-	-G0-	1
2	Ac-Lys-	-G1-	-G0-	1
3	Ac-Lys-	-G0/G1-	-G0-	1:1
4	Ac-Lys-	-G2-	-G0-	1
5	Ac-Lys-	-G0/G2-	-G0-	1:1
6	Ac-Lys-	-G1/G2-	-G0-	1:1
7	Ac-Lys-	-G0/G1/G2-	-G0-	1:1:1
8	Ac-Lys-	-G2-	-G1-	1
9	Ac-Lys-	-G0/G2-	-G1-	1:1
10	Ac-Lys-	-G0/G1-	-G2-	1:1
11	Ac-Lys-	-G1-	-G1-	1:1:1
12	Ac-Lys-	G0/G1/G2-	-G0/G1-	1:2:1
13	Ac-Lys-	-G0/G2-	-G0/G1-	1:1:1

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1	Ac-	-G1/G2-	-G0/G1-	1:2:1
4	Lys-			
1	Ac-	-	-G0/G1-	1:2:2:1
5	Lys-	G0/G1/G2-		
1	Ac-	-G2-	-G2-	1
6	Lys-			
1	Ac-	-G0/G2-	-G2-	1:1
7	Lys-			
1	Ac-	-G0/G2-	-G0/G2-	1:2:1
8	Lys-			
1	Ac-	-G1/G2-	-G2-	1:1
9	Lys-			
2	Ac-	-	-G2-	1:1:1
0	Lys-	G0/G1/G2-		
2	Ac-	-G1/G2-	-G0/G2-	1:1:1:1
1	Lys-			
2	Ac-	-	-G0/G2-	1:1:2:1:
2	Lys-	G0/G1/G2-		1
2	Ac-	-G1/G2-	-G1/G2-	1:2:1
3	Lys-			
2	Ac-	-	-G1/G2-	1:2:2:1
4	Lys-	G0/G1/G2-		
2	Ac-	-	-	1:2:3:2:
5	Lys-	G0/G1/G2-	G0/G1/G2-	1

Example 19

- 4 g of aminomethylated polystyrene (0.81 mmol/g) was put
 5 into each of 2-250 mL shakers. The resin was swelled with
 dimethylformamide (DMF) and drained. The resin was then
 washed with 20% piperidine/DMF for ~3 minutes and drained.
 The resin was washed three times with DMF, once with
 dichloromethane (DCM):DMF 1:1, and twice more with DMF.
 10 To the first shaker was added 3.5 equivalents of Knorr
 linker (6.12g, 11.34 mmol) and 3.5 equivalents of PyBop (5.9g,
 11.34 mmol). Enough DMF was added to cover the resin bed.
 The solution was then activated with 10.5 equivalents of DIEA
 (5.93 mL, 34.02 mmol). The reaction ran for 1 hour with
 15 manual shaking every 5-10 min.

To the second shaker was added 3.5 equivalents of photo
 linker (4.89g, 11.34 mmol) and 3.5 equivalents of PyBop (5.9g,

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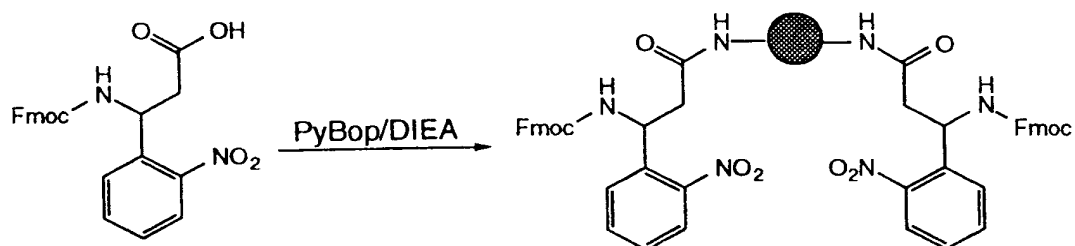
11.34 mmol). Enough DMF was added to cover the resin bed. The solution was then activated with 10.5 equivalents of DIEA (5.93 mL, 34.02 mmol). The reaction ran for 1 hour with manual shaking every 5-10 min.

5 After 1 hour both shakers were drained. Ninhydrin tests indicated complete coupling for both linkers. The resin was washed four times with DMF, once with dichloromethane (DCM):DMF 1:1, and two more times with DMF.

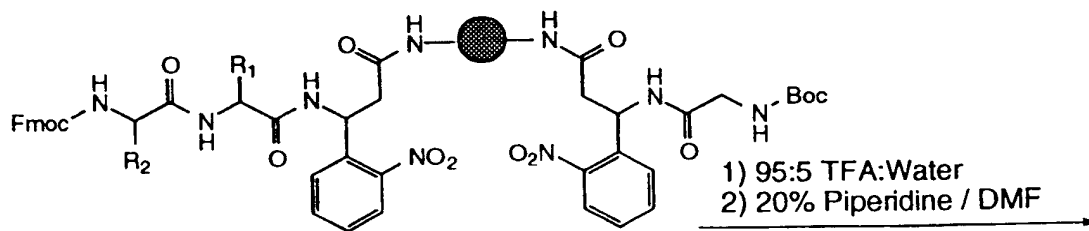
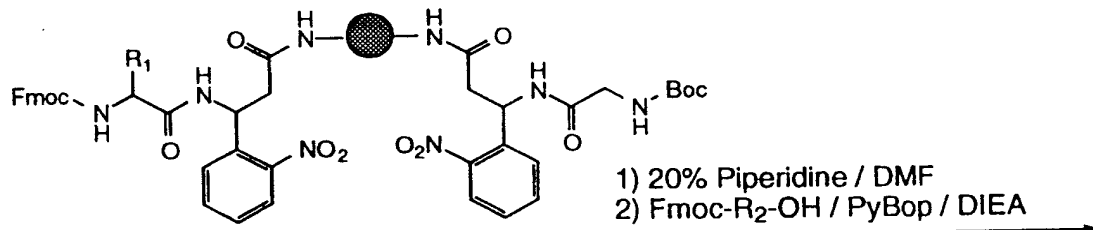
10 The resin was split to a 96 well ACT 496 Teflon reaction block.

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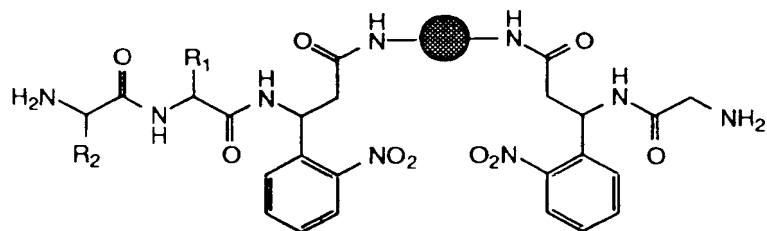
Example 20-Double Peak Positional Code



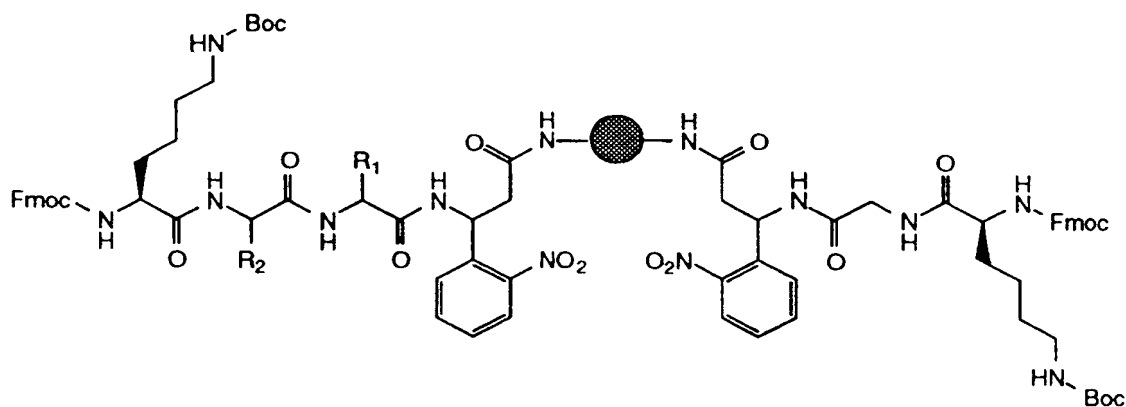
- 1) 20% Piperidine / DMF
 2) 1:1 Boc-Gly-OH : Fmoc-R₁-OH / PyBop / DIEA



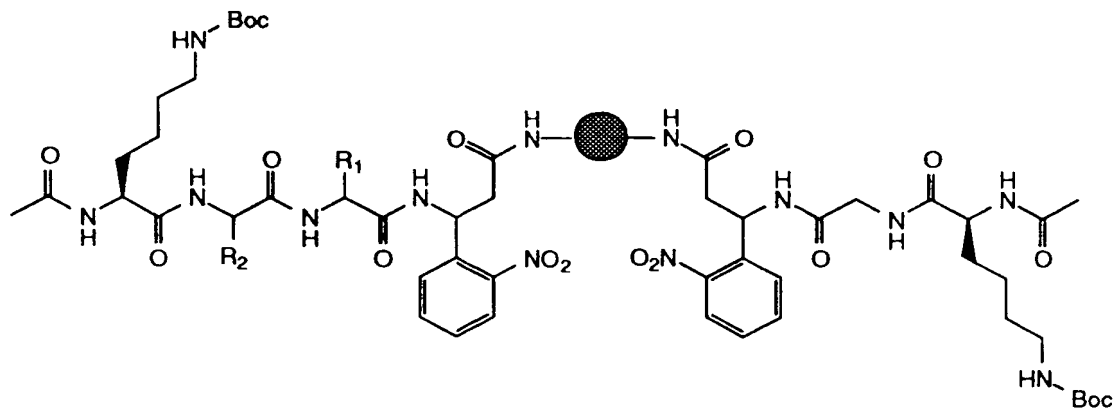
-82-



Fmoc-L-Lys(Boc)-OH / PyBop / DIEA

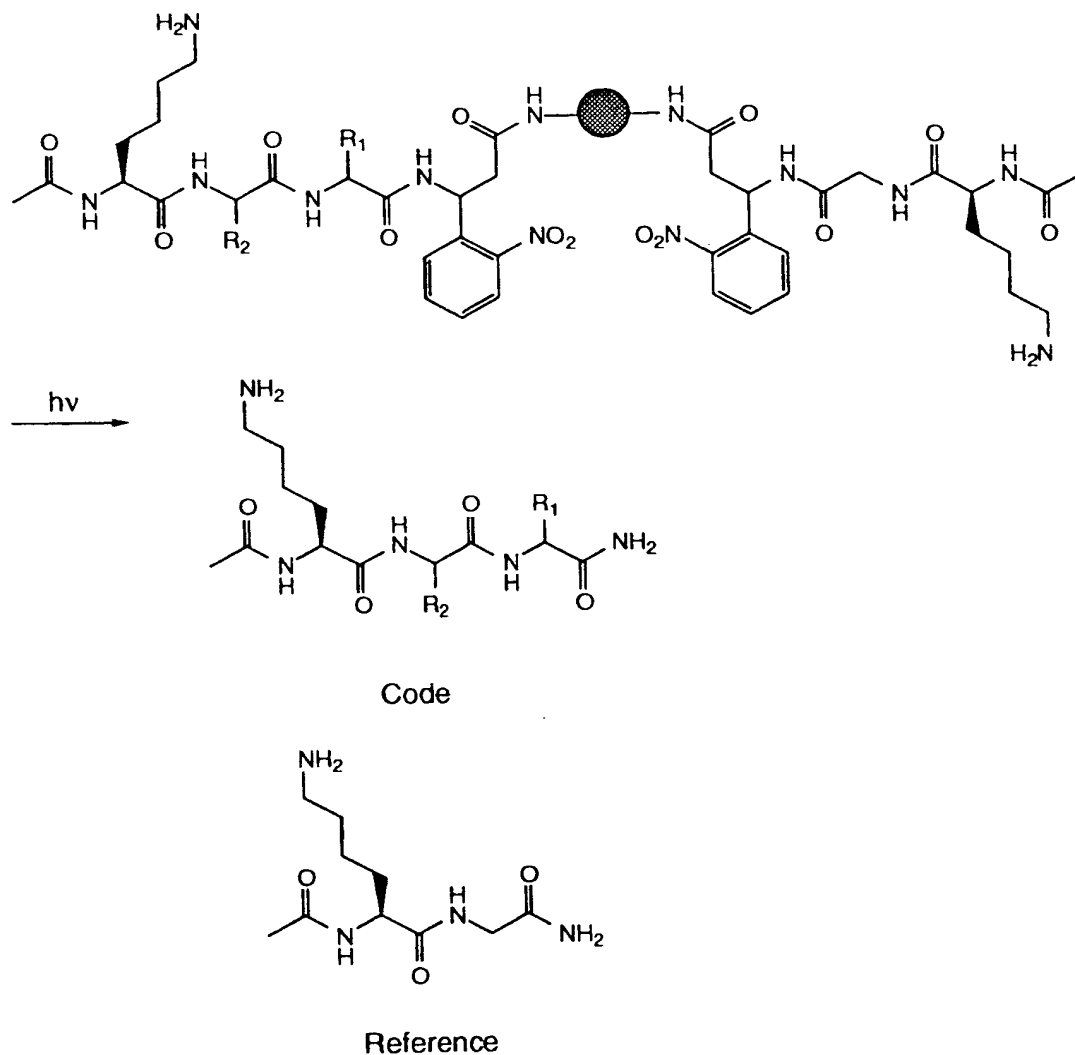


1) 20% Piperidine / DMF
2) 30% Acetic Anhydride / DMF



95:5 TFA:Water

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Example 21-Ratio Code Using Tertiary Amine

- 5 The seven lots of resin (CDW Fig. 2) were transferred to 10 mL shakers using dimethylformamide (DMF). The shakers were then covered with aluminum foil. The linker was Fmoc deprotected twice for 5 minutes each using 20% piperidine/DMF. The resin was washed four times with DMF, once with
- 10 dichloromethane (DCM):DMF 1:1, and twice with DMF.

0.315 mmol of each of seven halo acids were weighed in separate vials and an equimolar amount of PyBop was added to each. The halo acids were then dissolved in 2 mL of DMF. Each solution was then added to the respective lot of resin and activated

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with 165 μ L of diisopropylethylamine (DIEA). The coupling ran for 1 hour. After coupling the resins were drained and washed four times with DMF, once with DCM:DMF 1:1, and twice with DCM to dry the resin.

5 0.315 mmol of each of seven halo acids were weighed in separate vials and an equimolar amount of HBTU was added to each. The halo acids were then dissolved in 2 mL of DMF. Each solution was then added to the respective lot of resin and activated with 165 μ L of DIEA. The coupling ran for 1 hour. After
10 coupling the resins were drained and washed four times with DMF, once with DCM:DMF 1:1, and twice with DMF. Coupling was complete by ninhydrin.

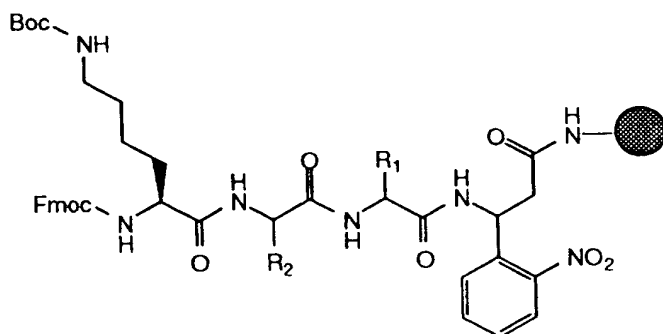
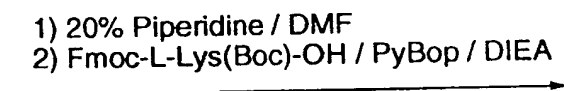
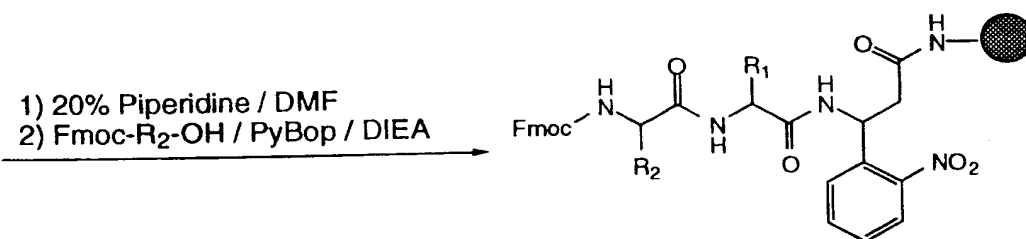
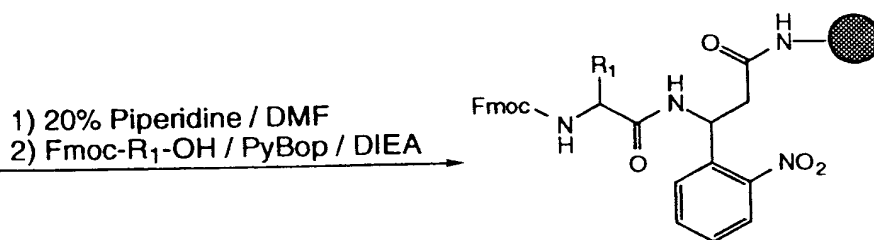
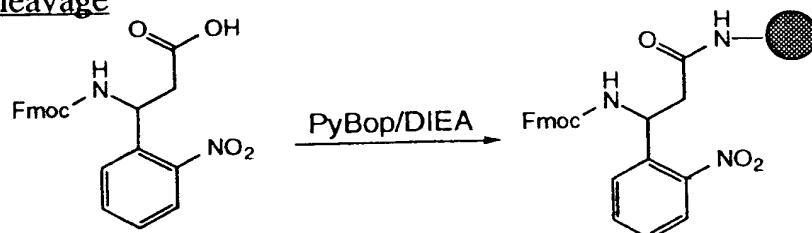
15 The resin lots were combined and split to 24 wells of a 96 well ACT 396 polypropylene reaction block. The resin was washed twice with DCM and dried.

20 The resin was swelled with DMF and drained. 1 mL of 1.0 M amine solution in dimethylsulfoxide (DMSO) was added to each well. The reaction ran overnight. The resin was drained and washed twice with DMF, once with DMF:methanol:water (1:1:1), and washed three more times with DMF. The 24 lots of resin were then combined and split back to 24 wells.

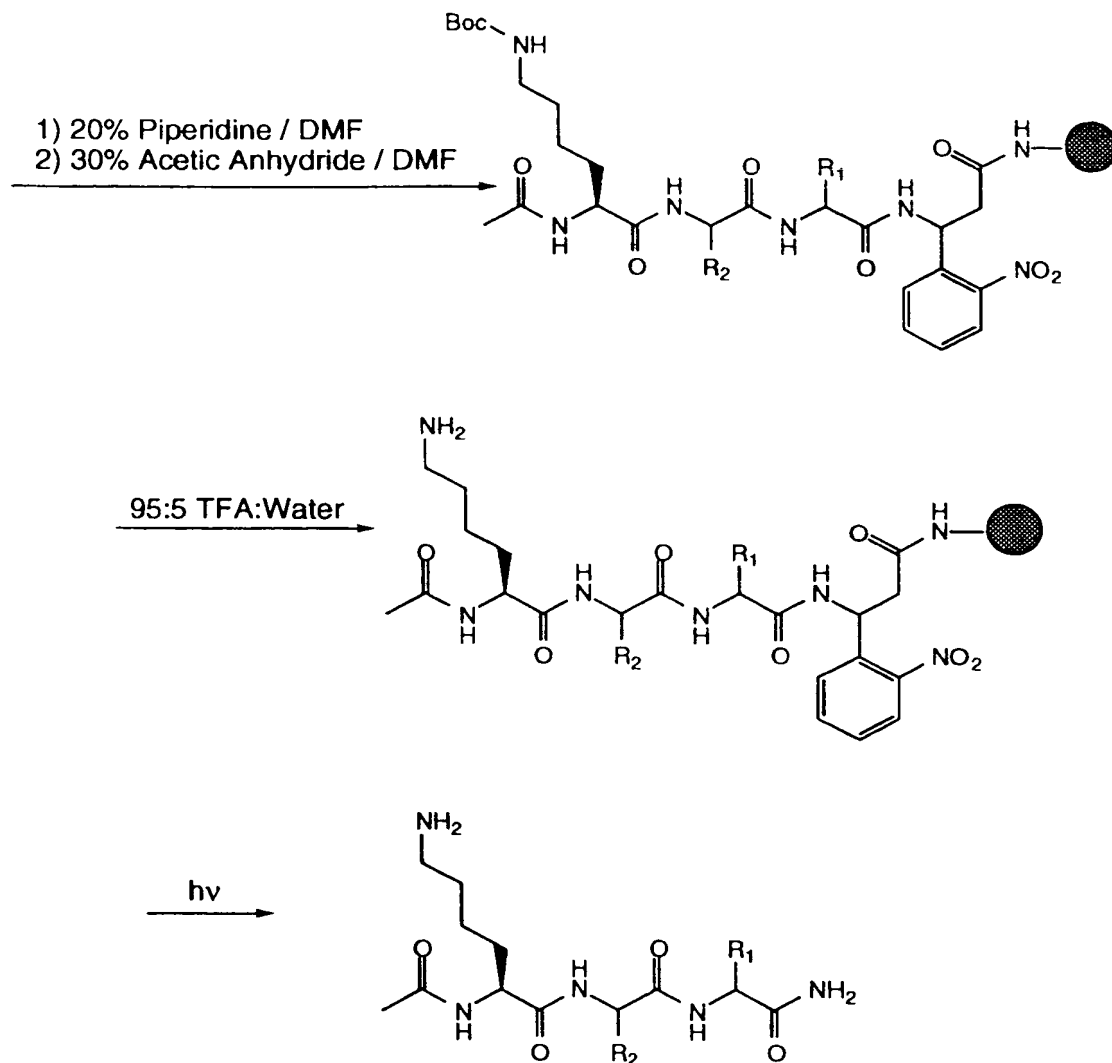
25 350 μ L of aldehyde solution (3.0 M / DMF) was added to each well and mixed for 2 minutes. An additional 250 μ L of trimethylorthoformate was added to each well and mixed for 1 minute followed by 100 μ L of 3% acetic acid in DMF. The reaction mixed for 15 minutes before a final addition of 1mL of 1.5 M NaBH₃CN. The reductive amination reaction ran for 3
30 hours. The resin was drained and washed twice with DMF.

35 A second reductive amination was run using sodium triacetoxymethylborohydride. 350 μ L of aldehyde solution (3.0 M / DMF) was added to each well and mixed for 2 minutes. An additional 250 μ L of trimethylorthoformate was added to each well and mixed for 1 minute followed by 100 μ L of 3% acetic acid in DMF. The reaction mixed for 15 minutes before a final
40 addition of 1mL of 1.5 M NaBH(O₂CCH₃). The reaction ran overnight. The resin was drained and washed twice with DMF, three times with DMF:methanol:water, three times with DCM, and three final times with methanol.

Example 22-Single Peak Positional Code With Photolabile Cleavage



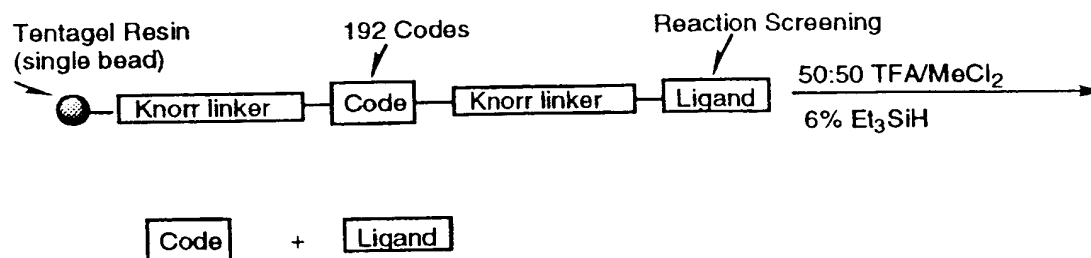
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Example 23-Reaction Screening

- 5 The synthesis of a set of serially encoded resins contained 192 combinations of isotopically labeled amino acids (glycine G^0 , G^1 , G^2 , G^3 and alanine: A^0 , A^1 , A^2 , and A^3) and were separated by Knorr linkers as shown below:

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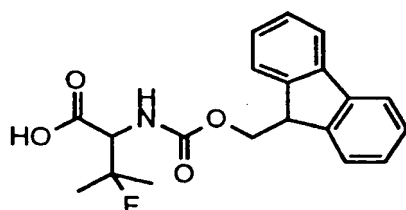
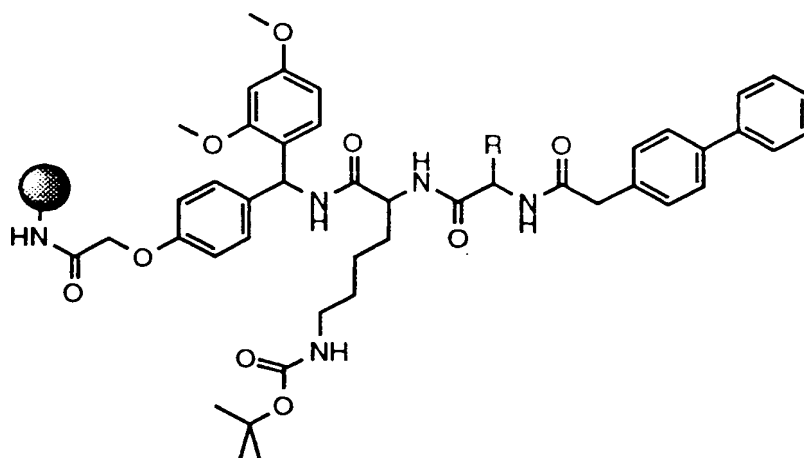
The reaction screening is performed on the ligand portion of the molecule where 3 different monomers were used for the coupling of
 5 primary amines to allylic bromides as well as 4 different reaction conditions to give a total of 12 combinations. The acylation of secondary amines with carboxylic acids involved 4 different acids and 4 different reaction conditions to give another 16 combinations. The resin was pooled and treated with 36 different conditions for the
 10 final intramolecular Heck reaction to give over 6900 potential compounds that contains different monomers and reaction conditions. Upon single bead cleavage with TFA/MeCl₂ and 6% triethylene both the encoded portion and the ligand are liberated as outlined above. The ligand is also encoded by the incorporation of
 15 glycine(0) and glycine(2) to help identify the ligand peaks in the mass spectrum.

The mass spectra that are shown in Figures 10 through 12 contain both the code (544-562) and the ligand (462 and 464), (504 and 506),
 20 (478 and 480). The monomers and conditions that were run for that particular mass spectrum are listed on the spectrum.

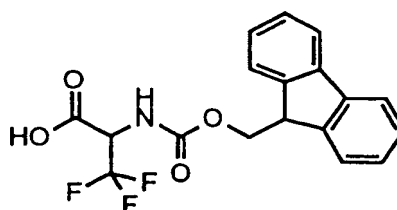
From these results one can use the combinatorial approach to look at various reaction conditions and monomers on a single bead and read
 25 both the code and the ligand when the material is cleaved off of the bead.

Example 24-Ratio Code; IR, NMR and MS analysis

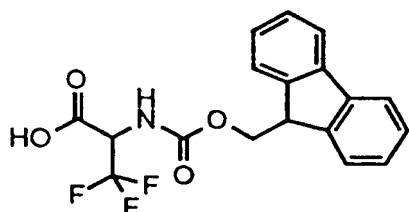
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Monomer 1 (R1)



Monomer 2 (R2)



Monomer 3 (R3)

16 resin samples were synthesized where R was defined by a simultaneous coupling of monomers 1 through 3 in different ratio patterns (ratios noted below). These resins could then be analysed by several analytical techniques to elucidate their ratio identity. The beads were analysed by IR, mass spec, NMR, and Amino Acid analysis. The ratios generated for the simultaneous coupling are

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- defined R1:R2:R3 and were as follows for the 16 samples: #1-1:1:5; Sample #2-1:2:5; Sample #3-1:3:5; Sample #4-1:4:5; Sample #5-2:1:5; Sample #6-2:2:5; Sample #7-2:3:5; Sample #8-2:4:5; Sample #9-3:1:5; Sample #10-3:2:5; Sample #11-3:3:5; Sample #12-3:4:5-
 5 Sample #13-4:1:5; Sample #14-4:2:5; Sample #15-4:3:5; Sample #16-4:4:5. Spectra for these ratios is shown in the accompanying Figures 18 through 51 as follows:

Sample	IR Spectra Number	Mass Spec. Number
1	u7125-86-1	CW-001
2	u7125-86-2	CW-002
3	u7125-86-3	CW-003
4	u7125-86-4	CW-004
5	u7125-86-5	CW-005
6	u7125-86-6	CW-006
7	u7125-86-7	CW-007
8	u7125-86-8	CW-008
9	u7125-86-9	CW-009
10	u7125-86-10	CW-0010
11	u7125-86-11	CW-0011
12	u7125-86-12	CW-0012
13	u7125-86-13	CW-0013
14	u7125-86-14	CW-0014
15	u7125-86-15	CW-0015
16	u7125-86-16	CW-0016

10 Example 25-Encoding Styrene

- The beads themselves generally used as solid supports in solid state synthesis can be isotopically doped. Styrene beads comprised of styrene can readily be doped with C¹³, F²⁰ or H².
 15 Additionally, it is possible to combine isotopically doped constructs with beads that have themselves been coded or tagged with one or more of a series of monomers.

- Styrene (M₀), 2-Fluorostyrene (M₁), 3-Fluorostyrene, (M₂) and 4-Fluorostyrene (M₃) were deinhibited prior to
 20 polymerization by passing through an alumina adsorption column.

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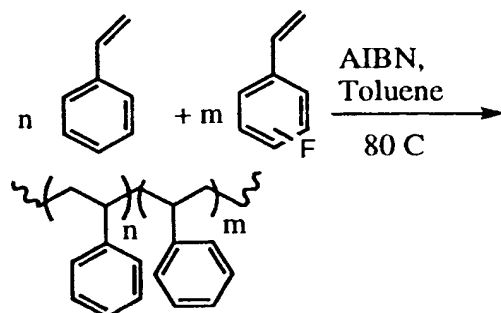
The initiator 2,2-azobisisobutyronitrile (AIBN) was purified by crystallization from methanol. Spectral grade toluene was used as solvent in a free radical polymerization without further purification.

5

Run	M ₁ (%)	M ₂ (%)	M ₃ (%)	M _s (%)
YL4001	100	0	0	0
YL4002	0	100	0	0
YL4003	0	0	100	0
YL4004	0	0	0	100
YL4005	0	0	50	50
YL4006	0	10	40	50
YL4007	0	20	30	50
YL4008	0	20	30	50
YL4009	0	40	10	50
YL4010	0	50	0	50
YL4011	50	0	0	50
YL4012	40	10	0	50
YL4013	30	20	0	50
YL4014	20	30	0	50
YL4015	10	40	0	50
YL4016	40	0	10	50
YL4017	30	0	20	50
YL4018	20	0	30	50
YL4019	10	0	40	50

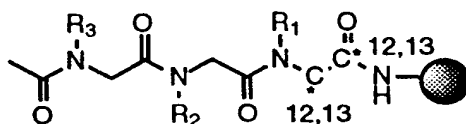
Copolymerization of Styrene and F-Styrene

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Example 26-Encoding a Peptoid Library

5



10 A 1000 component library consisting of three
monomer positions was designed to demonstrate the utility of the
encoding strategy. The library was synthesized using the split-mix
methodology to create a 10 x 10 x 10 library. Since the split mix
method yields one compound per bead and the beads are mixed
twice during the synthesis, the chemistry or monomer addition at
15 each step must be recorded on the bead in order to decode the
compound.

The first position is encoded by controlling the ratio
of $^{12}\text{C}:^{13}\text{C}_2$ incorporated with bromoacetic acid in the first
20 synthetic step. Ten unique ratios were employed with nine percent
increments ranging from 9:91 to 90:10. The use of nine percent
increments avoided the endpoints of either 0:100 or 100:0 while
allowing a total of ten codes. The third monomer was already
known because the library was not recombined after addition of
25 the final monomer (only one monomer from set three was present

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in each pool.) This position was therefore pool or spatially encoded.

The second position was encoded by the molecular weight of the compound. Since monomer one is known by the isotopic reaction and monomer three by the final pool, monomer two can be calculated using the molecular weight determined by the mass spectrometer. This imposes one constraint for any library: the monomers in the second position must have different molecular weights. The other monomers have no restrictions. For this test example, the final pools were composed of compounds having unique molecular weights in order to facilitate decoding.

Twenty single beads from each of the 10 pools were individually cleaved. The compounds were then analyzed by mass spectrometry and identified by the isotope ratio and molecular weight of the analyzed peaks. The peak resulting from the protonated monoisotopic ion was designated M_0H^+ while that resulting from the diisotopic ion (M_0H+2)⁺ was designated M_2H^+ . The deliberate doping with $^{13}C_2$ controlled the intensity of the M_2H^+ peak relative to the M_0H^+ peak.

Mass spectral data from a single bead in pool 3 showed that M_0H^+ and M_2H^+ were 552 and 554, respectively. The $M_0H^+ : M_2H^+$ ratio was calculated to be 10:90, which was quite close to the actual ratio for ion 552, which was 9:91. Using M_0H^+ as 550 and M_2H^+ as 552, the calculated M_0H^+ to M_2H^+ ratio was 56:44. The compound having a M_0H^+ ion in pool 2 had a theoretical of 54:46, again in very close agreement to the calculated ratio. In some cases, side-reactions involved in the addition of monomers may complicate the spectra. Spectra 2C below shows the mass spectrum of a single bead from pool 1. The most intense ion in the spectrum is at m/z 586. However, the calculated $M_0H^+ : M_2H^+$ ratio (65:35) does not agree with known ratio for the compound in pool 1 with M_0H^+ of 586. This apparent discrepancy is due to the addition of the third monomer for pool 1, a

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nitrile. It is known that under acidic conditions (cleavage conditions) nitriles can be hydrated to an amide. With the addition of water, the peaks for the M_0H^+ ion at 586 shift to 586 ($M_0H^+ + 18$). Thus, the $M_0H^+ : M_2H^+$ ratio for the peaks at 586 and 588 verifies the encode for the ion at 568 (which has its own, weaker set of peaks having the same ratio.). The known ratio for the compound in pool 1 with a M_0H^+ ion of 568 is 63:37, which corresponds to the calculated value. This particular hydration reaction was observed for all compounds in pool 1. This example demonstrates the ability to decode compounds and determine side reactions, a powerful enhancement over other encoding methodologies which would be very useful during solid phase chemistry development.

Example 27-Pseudo-Code Program Listing for Computer-Assisted
Detection of Isotope Encoded Compounds in a Mass Spectrum

1. Input the molecular formula and isotope ratios of an encoded compound.
2. Calculate the monoisotopic molecular weight from step 1.
3. Calculate the theoretical isotope distribution from step 1.
4. Open the mass spectrum output file.
5. Locate the monoisotopic molecular weight in the mass spectrum output file to within ± 0.3 amu and check for corresponding isotope peaks.
6. If step 5 was successful compare the theoretical distribution from step 3 with the measured values in the mass spectrum using a Chi-Square test. The outcome of the Chi-Square determines the presence or absence of the encoded compound.

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5 This invention is executable on a MacIntosh computer or a IBM-PC compatible computer, running the Windows 95, Windows NT or Macintosh OS operating system, which includes a CPU, main storage, I/O resources, and a user interface including a manually operated keyboard and mouse.

10

15

20 While the invention has been described and illustrated with reference to certain alternative embodiments thereof, those skilled in the art will appreciate that various changes, modifications and substitutions can be made therein without departing from the spirit and scope of the invention. It is intended, therefore, that the invention be limited only by the scope of the claims which follow, and that such claims be interpreted as broadly as possible.

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CLAIMS

What is claimed is:

- 5 1. A mass-based, non-chemical method for recording the reaction of at least a portion of a reaction series on each of a plurality of unique solid supports, said method comprising:
- 10 (a) preparing a plurality of agents each having a unique defined mass;
- (b) preparing a group of solid supports;
- 15 (c) reacting each solid support group with a different chemical reagent under a controlled reaction condition;
- 20 (d) mixing the product groups of step (c) together and then dividing said mixture of unique solid supports into a plurality of groups for a second intermediate or final stage;
- (e) repeating said reacting with a chemical reagent under a controlled reaction condition at least once to provide a plurality of final products, having different products on the different individual unique solid supports;
- 25 each of said unique defined mass agents being reacted with either: each of a group of unique solid supports; each of a group of first chemical reagents in a reaction series; each of a group of second chemical reagents in a reaction series; or each of a group of subsequently added chemical reagents in a reaction series; such
- 30 that each of said group of unique solid supports, group of first chemical reagents, group of second chemical reagents or group of subsequent chemical reagents has been reacted with an agent

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having a defined mass that is different from any other defined mass agent reacted with any other of said groups;

5 said unique defined mass agents being capable of being analyzed and wherein said analysis defines the choice of a first chemical reagent, reaction condition under which said first chemical reagent was added, second chemical reagent, reaction condition under which said second chemical reagent was added, subsequent chemical reagent, or reaction condition under which
10 said subsequent chemical reagent was added.

2. A mass-based, non-chemical method for recording the reaction history of a reaction series on each of a plurality of unique solid supports, said method comprising:

15

 (a) reacting, at a mass block insertion stage, a plurality of agents each having a unique defined mass with each of a group of said unique solid supports, such that each of said group of unique solid supports has been reacted with an agent having a
20 defined mass that is different from any other agent reacted with any other of said groups of said unique solid supports;

 (b) reacting each solid support group having a different defined mass agent with a different chemical reagent;

25

 (c) mixing said groups together and then dividing said plurality of unique solid supports into a plurality of groups for a second intermediate or final stage;

30 (d) repeating said reacting with a chemical reagent at least once to provide a plurality of final products, having different products on the different individual unique solid supports;

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said unique defined mass agents being capable of being analyzed and wherein said analysis defines the choice of a first chemical reagent.

- 5 3. The method as claimed in claim 1, wherein said defined mass agents are analyzed by mass spectroscopy.
4. The method as claimed in claim 3, wherein said defined mass agents are selected so as to each generate a unique single
10 mass peak when analyzed by mass spectroscopy.
5. The method as claimed in claim 3, wherein said defined mass agents are selected so as to each generate a unique double mass peak when analyzed by mass spectroscopy.
- 15 6. The method as claimed in claim 3, wherein said defined mass agents are selected so as to each generate a unique pair of single mass peaks when analyzed by mass spectroscopy.
- 20 7. The method as claimed in claim 3, wherein said defined mass agents are selected so as to each generate a unique pair of double mass peaks when analyzed by mass spectroscopy.
8. The method as claimed in claim 3, wherein said defined
25 mass agents are selected so as to each generate a unique pattern of one or more mass peaks.
9. The method as claimed in claim 8, wherein said unique peak patterns for each of said defined mass agents can be
30 expressed as a machine-readable pattern.
10. The method as claimed in claim 9, wherein said machine readable patterns are bar codes.

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11. The method as claimed in claim 3, wherein said defined mass agents are selected so as to each independantly generate a unique mass spectrometry mass peak pattern selected from the group consisting of unique single mass peaks, unique double mass peaks, unique pairs of single mass peaks, unique pairs of double mass peaks, and unique peak patterns that are capable of being expressed as machine-readable patterns.
12. The method as claimed in claim 1, wherein said defined mass agent is analyzed by nuclear magnetic resonance spectroscopy.
13. The method as claimed in claim 12, wherein said defined mass agents are selected so as to each generate a unique pattern of one or more nuclear magnetic resonance peaks.
14. The method as claimed in claim 13, wherein said unique peak patterns for each of said defined mass agents can be expressed as a machine-readable pattern.
15. The method as claimed in claim 14, wherein said machine readable patterns are bar codes.
16. The method as claimed in claim 1, wherein said defined mass agent is analyzed by infrared spectroscopy or by Raman spectroscopy.
17. The method as claimed in claim 16, wherein said defined mass agents are selected so as to each generate a unique pattern of one or more infrared spectroscopy or Raman spectroscopy peaks.
18. The method as claimed in claim 17, wherein said unique peak patterns for each of said defined mass agents can be expressed as a machine-readable pattern.

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19. The method as claimed in claim 18, wherein said machine readable patterns are bar codes.
- 5 20. The method as claimed in claim 1, wherein said first, second or subsequent reagent is a substrate for the determination of binding specificity to a chemical compound of interest.
- 10 21. The method as claimed in claim 3, wherein said mass spectroscopy analysis provides mass peaks capable of being recognized as representing encoded reagents.
- 15 22. The method as claimed in claim 1, wherein additional mass peaks are generated that serve as signature peaks for positive identification of relevant mass peaks.
- 20 23. The method as claimed in claim 1, wherein said plurality of defined mass agents are molecular entities that differ from one another by having at least one of their atoms substituted by a different isotope of that atom, provided that the chemical structural formula of said defined mass agents is the same.
- 25 24. The method as claimed in claim 23, wherein said plurality of defined mass agents are molecular entities that differ from one another by having at least one isotopic substitution at different atomic positions within the molecule provided that the chemical structural formula of said defined mass agents is the same.
- 30 25. The method as claimed in claim 1, wherein said plurality of defined mass agents are regularly repeating molecular entities that differ from one another by an integral number of said repeating molecular entities.

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26. The method as claimed in claim 1, wherein at least two groups of said unique solid supports are employed in each said reacting.
- 5 27. The method as claimed in claim 1, comprising the additional step of screening said final products on said unique solid supports for a characteristic of interest and identifying the reaction history of at least one final product having said characteristic of interest.
- 10 28. The method as claimed in claim 1, comprising the additional step of cleaving said final products from said solid supports and screening said final products.
- 15 29. The method as claimed in claim 1, wherein said analysis is automated.
30. The method as claimed in claim 10, wherein said reaction steps are automated.
- 20 31. A kit for encoding the reaction history of a plurality of reaction series, comprising a plurality of different isotopically distinguishable organic compounds, each of the compounds characterized by having distinguishable masses but having the same chemical composition and the same chemical properties,
- 25 each compound encoding at least one bit of different physical information which can be determined by a physical measurement.
- 30 32. A kit as claimed in claim 31, wherein said compounds are mixed with one another in a plurality of discrete ratios to produce a plurality of isotope mixtures that are physically distinguishable from each other.

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33. A kit as claimed in claim 32, wherein said compounds are mixed with one another in a series of regularly repeating increasing increments.

- 5 34. A kit as claimed in claim 31, wherein said compounds are of the formula:

R-C

- 10 where R is a suitable solid support which allows for attachment and detachment of a molecular moiety of choice; and C is an isotopically doped linker which allows for attachment and detachment from said solid support.

- 15 35. A kit as claimed in claim 31, wherein said compounds are of the formula:

L^1 -C- L^2

- 20 where L^1 is a covalent bond or an organic moiety; C is an isotopically doped linker; and L^2 is a covalent bond or an organic moiety.

- 25 36. A kit as claimed in claim 35, wherein L^1 and L^2 are the same.

37. A kit as claimed in claim 31, wherein said compounds are of the formula

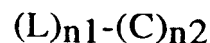
30 R- L^1 -C- L^2

where R is a suitable solid support which allows for attachment and detachment of a molecular moiety of choice; L^1 is a covalent

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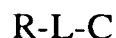
bond or an organic moiety; C is an isotopically doped linker; and L² is a covalent bond or an organic moiety.

38. A kit as claimed in claim 31, wherein said compounds are
5 of the formula



10 where n₁ is an integer of from one to ten, n₂ is an integer of from one to ten, L an organic moiety or a covalent bond when n₁ is one, and C is an isotopically doped linker.

39. A kit as claimed in claim 31, wherein said compounds are
15 of the formula



20 where R is a suitable solid support which allows for attachment and detachment of a plurality of molecular moiety of choice; L is a covalent bond or an organic moiety; and C is an isotopically doped linker.

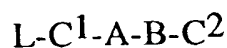
40. A kit as claimed in claim 31, wherein said compounds are
25 of the formula



30 L is a covalent bond or an organic moiety; C¹ is an isotopically doped linker; A is a first monomer in a reaction series, B is a second monomer in a reaction series, and D is a third monomer in a reaction series; and C² is a second isotopically doped linker that can physically be the same as or different from C¹.

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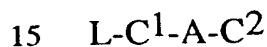
41. A kit as claimed in claim 31, wherein said compounds are of the formula



5

L is a covalent bond or an organic moiety; C^1 is an isotopically doped linker; A is a first monomer in a reaction series, B is a second monomer in a reaction series; and C^2 is a second isotopically doped linker that can physically be the same as or
10 different from C^1 .

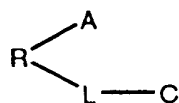
42. A kit as claimed in claim 31, wherein said compounds are of the formula



L is a covalent bond or an organic moiety; C^1 is an isotopically doped linker; A is a first monomer in a reaction series; and C^2 is a second isotopically doped linker that can physically be the same
20 as or different from C^1 .

43. A kit as claimed in claim 31, wherein said components are isotopically doped suitable solid supports.

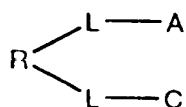
25 44. A kit as claimed in claim 31, wherein said components are of the formula



30 where R is a suitable solid support; L is a covalent bond or an organic moiety; C is an isotopically doped moiety; and A is a first chemical reagent in a reaction series.

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45. A kit as claimed in claim 31, where said components are of the formula



5

where R is a suitable solid support; L is a covalent bond or an organic moiety; C is an isotopically doped moiety; and A is a first chemical reagent in a reaction series.

10 46. A solid support characterized by having a unique defined mass agent and a ligand bound to the surface of said solid support.

47. A solid support characterized by having up to 20 discrete unique defined mass agents bound to the surface of said solid
15 support.

48. A solid support as claimed in claim 46, wherein said ligand is an organic moiety.

20 49. A solid support as claimed in claim 46, wherein said ligand is bound to said unique defined mass agent.

50. A solid support as claimed in claim 46, wherein said ligand is a non-oligomer which is aliphatic, alicyclic, aromatic,
25 heterocyclic or a combination thereof.

51. A solid support as claimed in claim 46, wherein said ligand is an oligomer which is an oligopeptide, oligonucleotide, oligosaccharide, poly lipid, polyester, polyamide, polyurethane,
30 polyurea, polyether, polyphosphorus where phosphorus is a derivative taken from the group consisting of phosphate, phosphonate, phosphoramidate, phosphonamide, phosphite, or phosphinamide, or polysulfur where sulfur is a derivative taken

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from the group consisting of sulfone, sulfonate, sulfite, sulfinamide, or sulfenamide.

52. A solid support as claimed in claim 46, wherein said
5 support is a resin bead of about 1 to 10000 μm in diameter.

53. A solid support as claimed in claim 46, wherein said
support is a polystyrene resin bead of about 1 to 10000 μm in
diameter.

10 54. A solid support characterized by being isotopically doped.

55. A solid support as claimed in claim 54, wherein said
support is an isotopically doped resin bead.

15 56. A solid support as claimed in claim 55, wherein said
support is an isotopically doped polystyrene resin bead of about
10 to 2000 μm in diameter.

20 57. A library comprising a plurality of solid supports as
claimed in claim 46.

58. A library comprising a plurality of solid supports as
claimed in claim 46, wherein said final products have been
25 cleaved from said solid supports.

59. A process for identifying compounds having a
characteristic of interest, which comprises screening a library as
claimed in claim 57.

30 60. A process as claimed in claim 59, wherein the compounds
have been cleaved from the solid support.

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61. A process as claimed in claim 60, wherein said cleavage is between said solid support and said unique defined mass agent.

5 62. A mass-based, non-chemical method for generating machine or human-recognizable patterns to record the reaction history of a reaction series on each of a plurality of unique solid supports, said method comprising:

10 (a) generating a set of agents each having a unique defined mass such that each agent differs from any other agent in the set by having a defined mass that is different from any other agent in the set;

15 (b) generating a recognition pattern for each agent in said set;

20 (c) reacting, at a mass block insertion stage, a plurality of agents each having a unique defined mass with each of a group of said unique solid supports, such that each of said group of unique solid supports has been reacted with an agent having a defined mass that is different from any other agent reacted with any other of said groups of said unique solid supports;

25 (d) reacting each solid support group having a different defined mass agent with a different first chemical reagent;

30 (e) mixing said groups together and then dividing said plurality of unique solid supports into a plurality of groups for a second intermediate or final stage;

(f) optionally repeating said reacting with a chemical reagent at least once to provide a plurality of final products, having different products on the different individual unique solid supports;

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(g) analyzing said products for a characteristic of interest;

5 (h) further analyzing products found to have a characteristic of interest in step (g) by an analytical method that generates a like type of patterns as that type generated for the recognition patterns in step (b); and

10 (i) comparing the analytical patterns generated in step (h) to said recognition patterns.

63. A mass-based, non-chemical method for generating machine-recognizable patterns to record the reaction history of a
15 reaction series on each of a plurality of unique solid supports, said method comprising:

(a) generating a set of agents each having a unique defined mass such that each agent differs from any other agent in
20 the set by having a defined mass that is different from any other agent in the set;

(b) generating a machine-recognizable recognition pattern for each agent in said set;

25 (c) reacting, at a mass block insertion stage, a plurality of agents each having a unique defined mass with each of a group of said unique solid supports, such that each of said group of unique solid supports has been reacted with an agent having a
30 defined mass that is different from any other agent reacted with any other of said groups of said unique solid supports;

(d) reacting each solid support group having a different defined mass agent with a different first chemical reagent;

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(e) mixing said groups together and then dividing said plurality of unique solid supports into a plurality of groups for a second intermediate or final stage;

5

(f) optionally repeating said reacting with a chemical reagent at least once to provide a plurality of final products, having different products on the different individual unique solid supports;

10

(g) analyzing said products for a characteristic of interest through the use of an analysis device;

(h) further analyzing products found to have a characteristic of interest in step (g) by an analytical method that generates a like type of patterns as that type generated for the recognition patterns in step (b); and

15

(i) comparing the analytical patterns generated in step (h) to said recognition patterns such that said unique defined mass agents are capable of being analyzed and identified.

20

64. The method as claimed in claim 63, wherein said identification in step (i) leads to the ready identification of said first chemical reagent.

25

65. The method as claimed in claim 63, wherein said method steps are executed by suitable automation apparatus means and under the control of a suitable computer means.

30

66. The method as claimed in claim 63, wherein said analysis device is selected from the group consisting of a fluorescence activation cell scanner, a chromatography column or a chromatography plate.

-109-

67. The method as claimed in claim 63, wherein said analytical method is selected from the group consisting of mass spectrometry, nuclear magnetic resonance spectroscopy, infrared spectroscopy or Raman spectroscopy.

68. The method as claimed in claim 63 wherein said analysis device is close coupled to said automation apparatus used in the method so as to be automated and under the control of a computer.

69. The method as claimed in claim 63, wherein said analytical method is executed by a corresponding device that is close coupled to said automation apparatus used in the method so as to be automated and under the control of a computer.

70. A database readily retrievable from a suitable data storage means comprised of the set of machine-readable patterns generated by the method as claimed in claim 63.

71. A mass-based, non-chemical method for generating machine or human-recognizable patterns to record the reaction history of a reaction sequence of interest selected from a reaction series on each of a plurality of unique solid supports, said method comprising:

(a) generating a set of agents each having a unique defined mass such that each agent differs from any other agent in the set by having a defined mass that is different from any other agent in the set;

(b) generating a recognition pattern for each agent in said set;

-110-

(c) reacting, at a mass block insertion stage, a plurality of agents each having a unique defined mass with each of a group of said unique solid supports, such that each of said group of unique solid supports has been reacted with an agent having a defined mass that is different from any other agent reacted with any other of said groups of said unique solid supports;

(d) reacting each solid support group having a different defined mass agent with a different first chemical reagent;

10

(e) mixing said groups together and then dividing said plurality of unique solid supports into a plurality of groups for a second intermediate or final stage;

15

(f) optionally repeating said reacting with a chemical reagent at least once to provide a plurality of final products, having different products on the different individual unique solid supports;

20

(g) analyzing said products for a characteristic of interest;

(h) further analyzing products found to have a characteristic of interest in step (g) by an analytical method that generates a like type of patterns as that type generated for the recognition patterns in step (b);

25

(i) comparing the analytical patterns generated in step (h) to said recognition patterns;

30

(j) evaluating said analytical patterns to arrive at a qualitative and quantitative assessment of the output of a reaction sequence of interest, thereby identifying all products, quantities

-111-

and yields of each of incomplete reactions, side reactions and previously unknown reactions in said sequence of interest.

72. A mass-based, non-chemical method for recording the
5 reaction history of a reaction series in solution, said method comprising:

(a) preparing, a plurality of agents each having a unique
defined mass within each of a group of solution reaction wells,
10 such that each of said group of reaction wells contains an agent having a defined mass that is different from any other agent within any other of said reaction wells;

(b) reacting each different defined mass agent with a
15 different first chemical reagent in each well;

(c) mixing said groups together in to a resulting batch
and then dividing said batch into a plurality of wells for a second
intermediate or final stage;

20 (d) repeating said reacting with a chemical reagent at least once to provide a plurality of final products, having different products within said wells;

25 said unique defined mass agents being capable of being analyzed and wherein said analysis defines the choice of said first chemical reagent.

73. A programmed computer system for executing a mass-
30 based method for recording the reaction history of at least a portion of a reaction series of interest on each of a plurality of solid supports or in each or a plurality of reaction vessels, wherein one or more chemical reagents or chemical conditions are discretely identifiable by one or more recognition patterns

-112-

and wherein said reaction series' chemical products are subjected to analytical means that generate analytical patterns for each of said products; comprising:

5 first input means for introducing unique recognition patterns into the computer system, each pattern representing one of a plurality of agents each having a unique defined mass;

10 memory means for storing said recognition pattern;

 second input means for introducing said resultant analytical patterns; and

15 means for comparing said resulting analytical patterns to said recognition patterns in order to generate an output which is the identity of one or more of said chemical reagents or chemical conditions.

74. The programmed computer system as claimed in claim 73,
20 additionally comprising means for controlling a robot means for performing one or more steps of said reaction series of interest.

75. The programmed computer system as claimed in claim 73,
25 additionally comprising means for generating said unique recognition patterns.

76. The programmed computer system as claimed in claim 73,
30 additionally comprising means for subjecting said reaction products to analytical means and generating analytical patterns.

77. A mass-based, non-chemical method for identifying different chemical compounds in a mixture of said chemical compounds, said method comprising:

-113-

(a) preparing a plurality of chemical agents each having a unique defined mass;

5 (b) preparing a group of chemical compounds to be identified;

each of said unique defined mass agents being chemically linked or reacted with each of said chemical compounds to be identified to form a plurality of unique covalently bound one-to-one pairs of unique defined mass agents with compounds to be identified;

10

said unique defined mass agents being capable of being analyzed on the basis of its mass, and wherein said analysis thus identifies the chemical compound reacted with said analyzed mass agent.

15

78. A mass-based, non-chemical method for identifying a chemical compound, said method comprising:

20

(a) preparing a chemical agent having a unique defined mass;

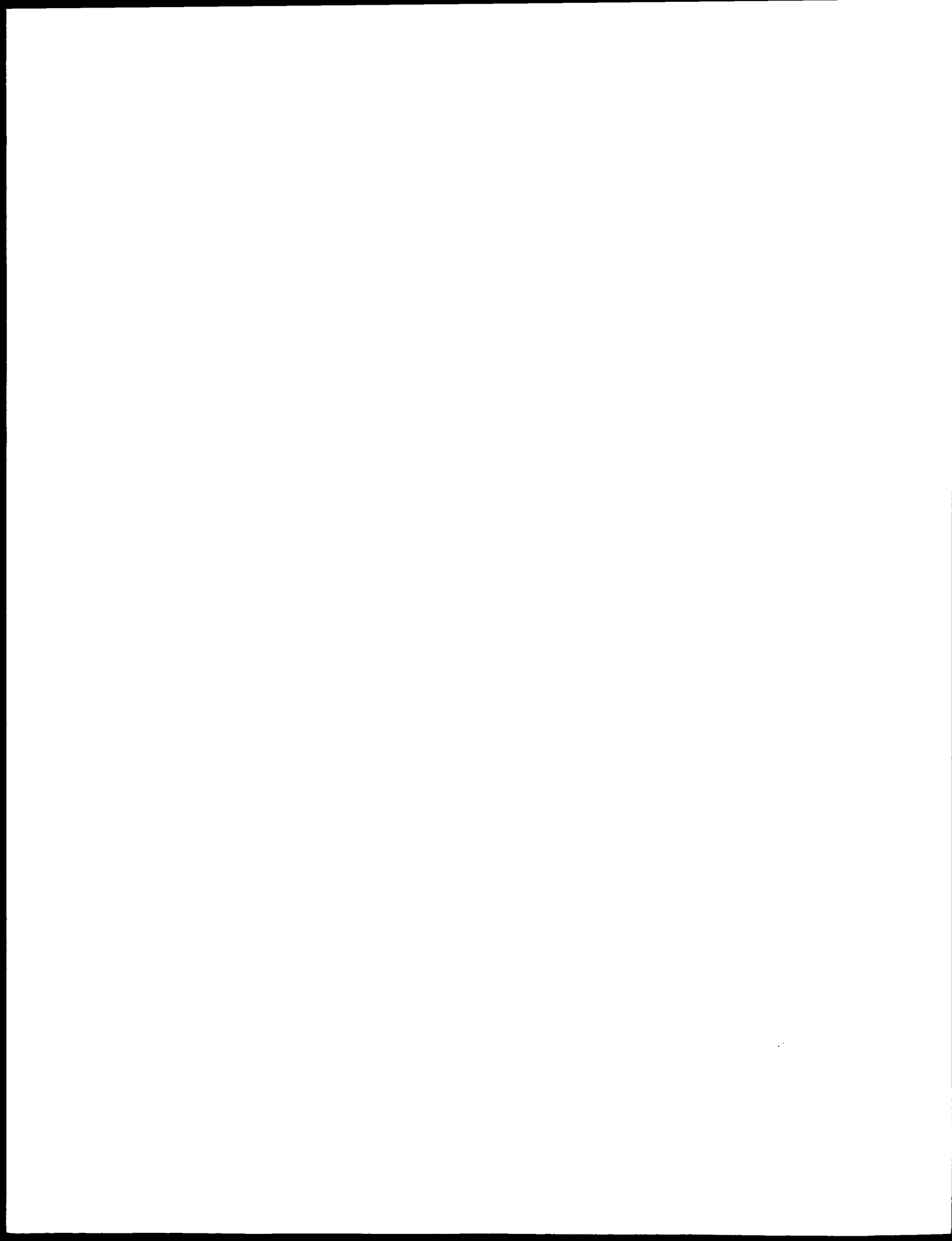
(b) preparing a chemical compound to be identified;

25

said unique defined mass agent being chemically linked or reacted with said chemical compound to be identified to form a unique covalently bound one-to-one pairing of unique defined mass agent with compound to be identified;

30

said unique defined mass agent being capable of being analyzed on the basis of its mass, and wherein said analysis thus identifies the chemical compound reacted with said analyzed mass agent.



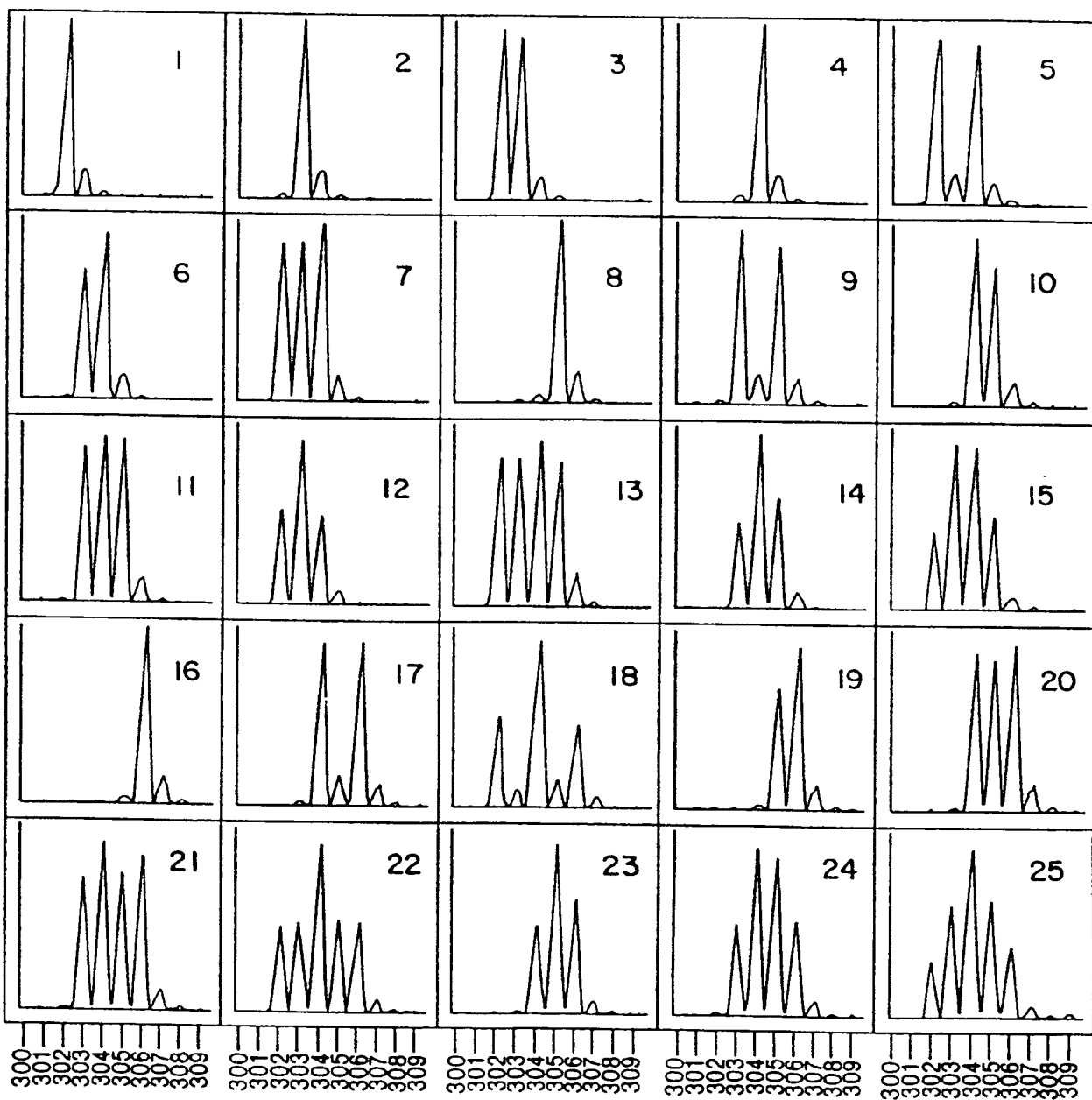


FIG 1

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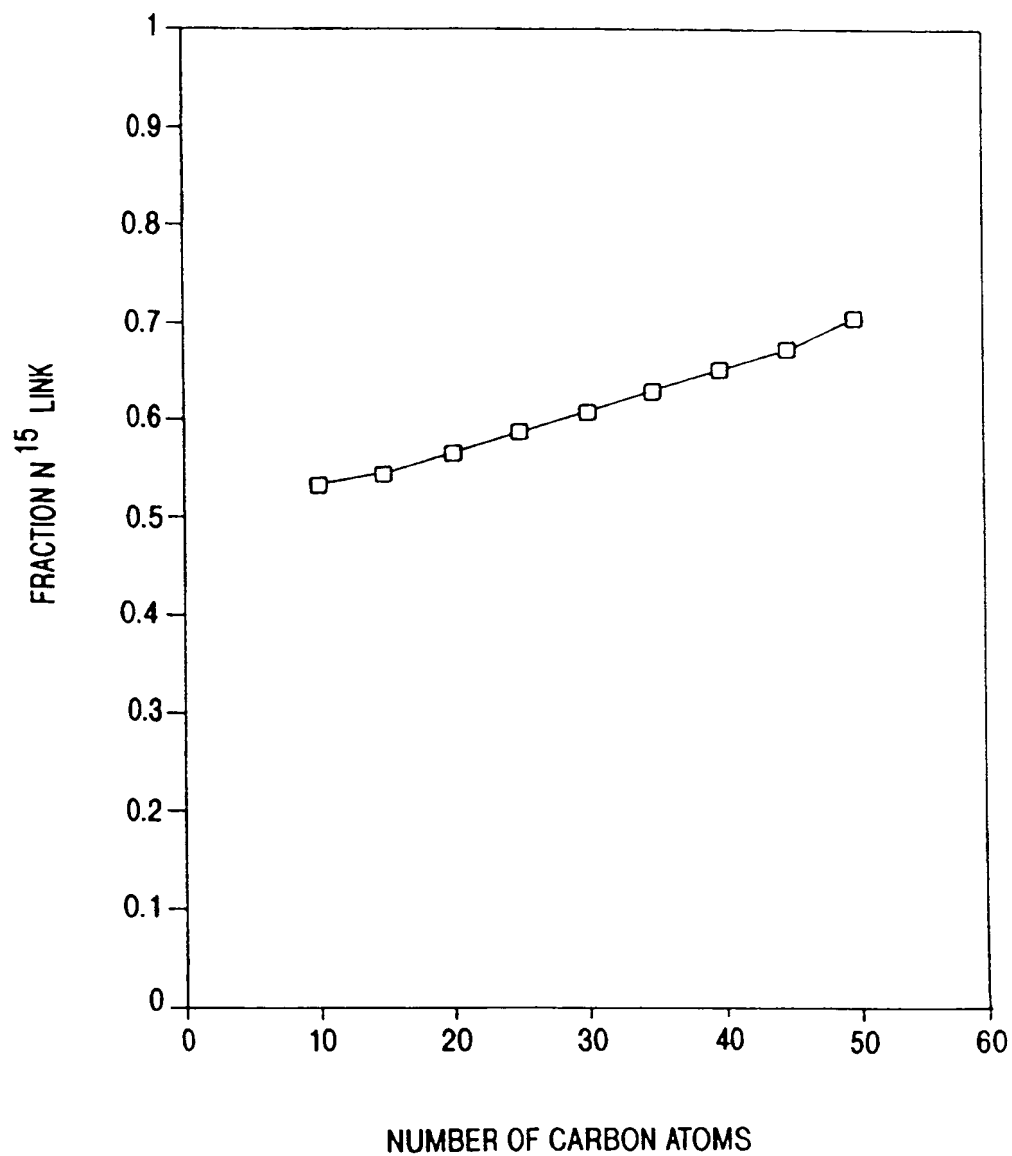
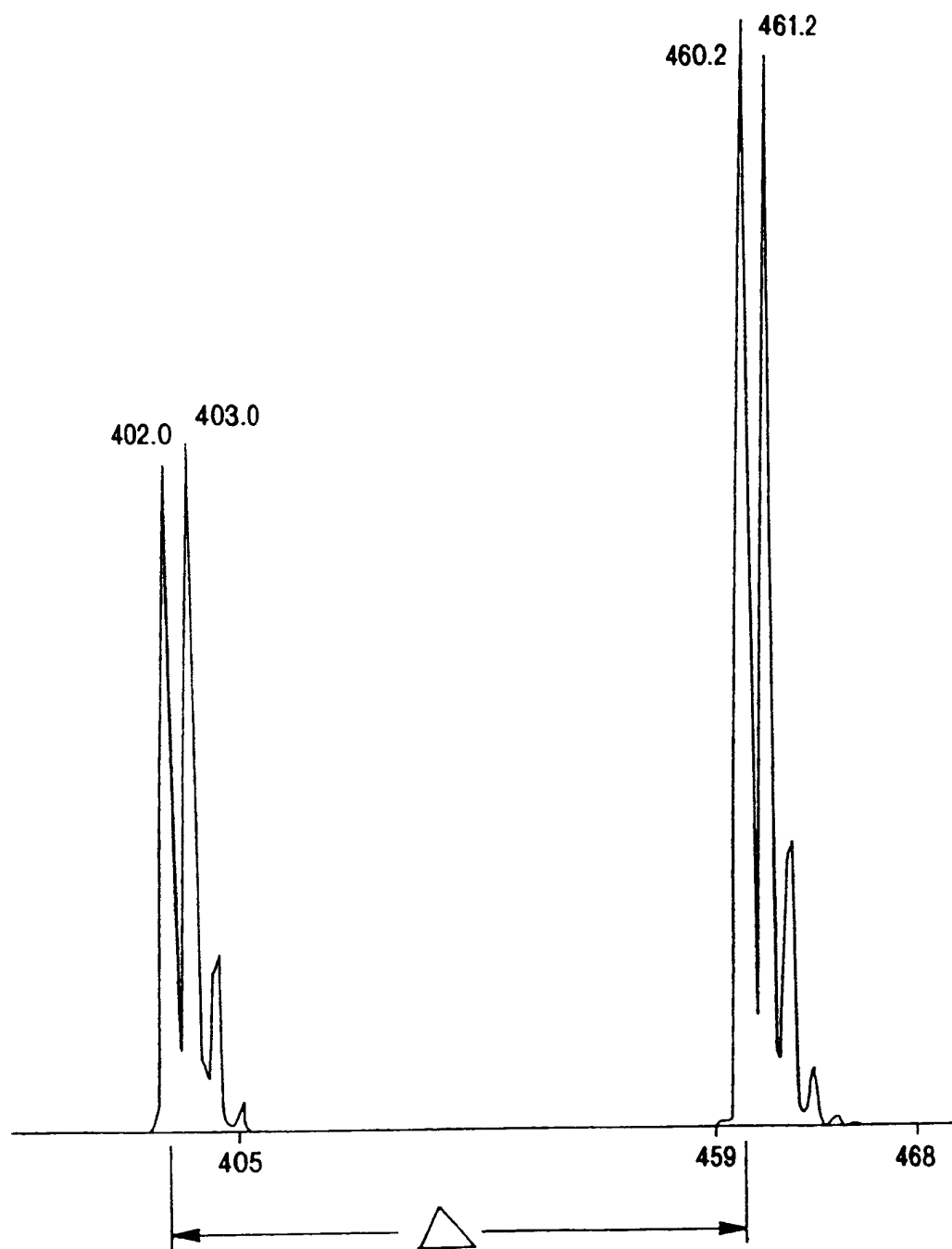


FIG. 2

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CODE n

FIG. 3

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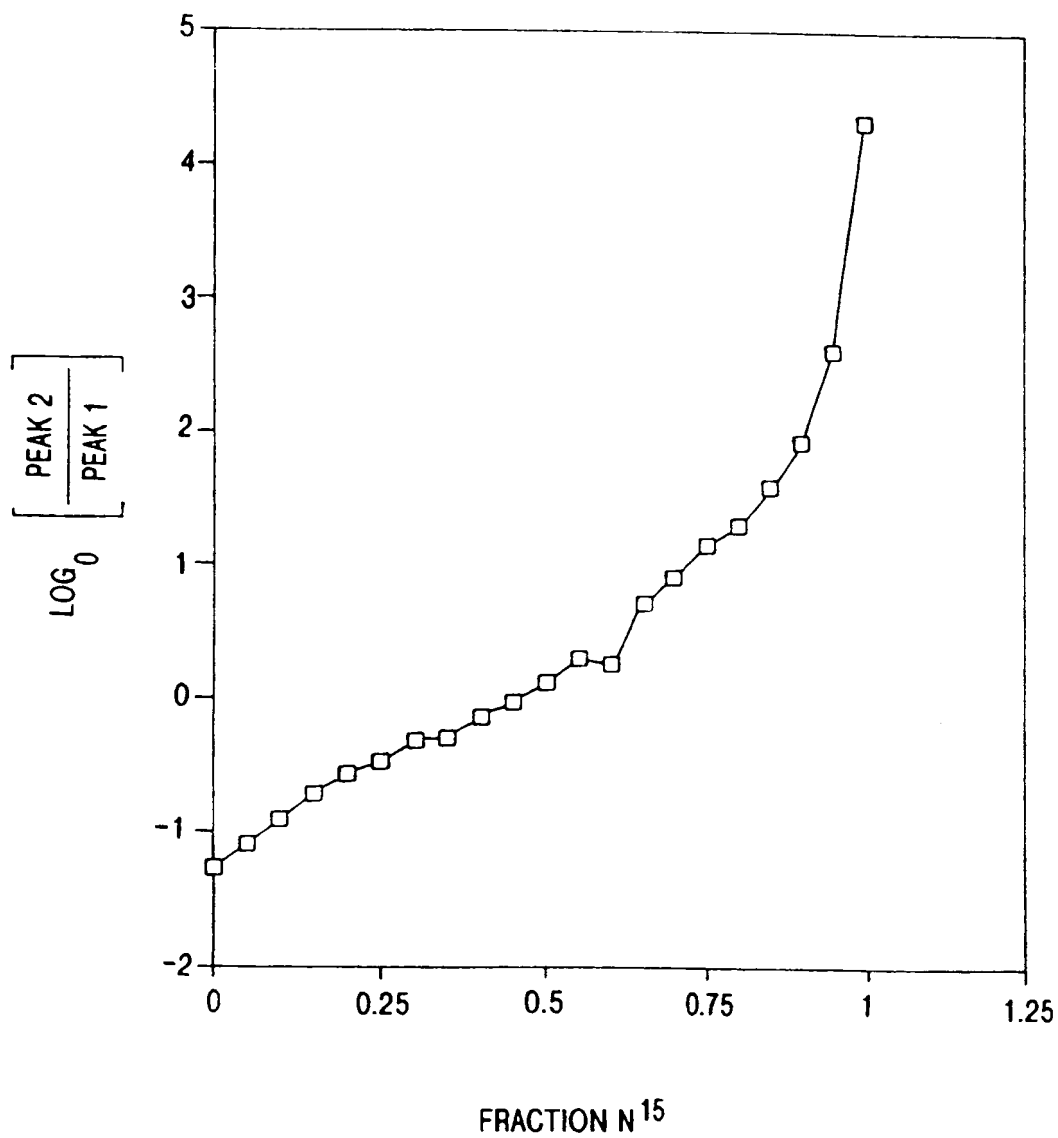
FRACTION N¹⁵

FIG. 4

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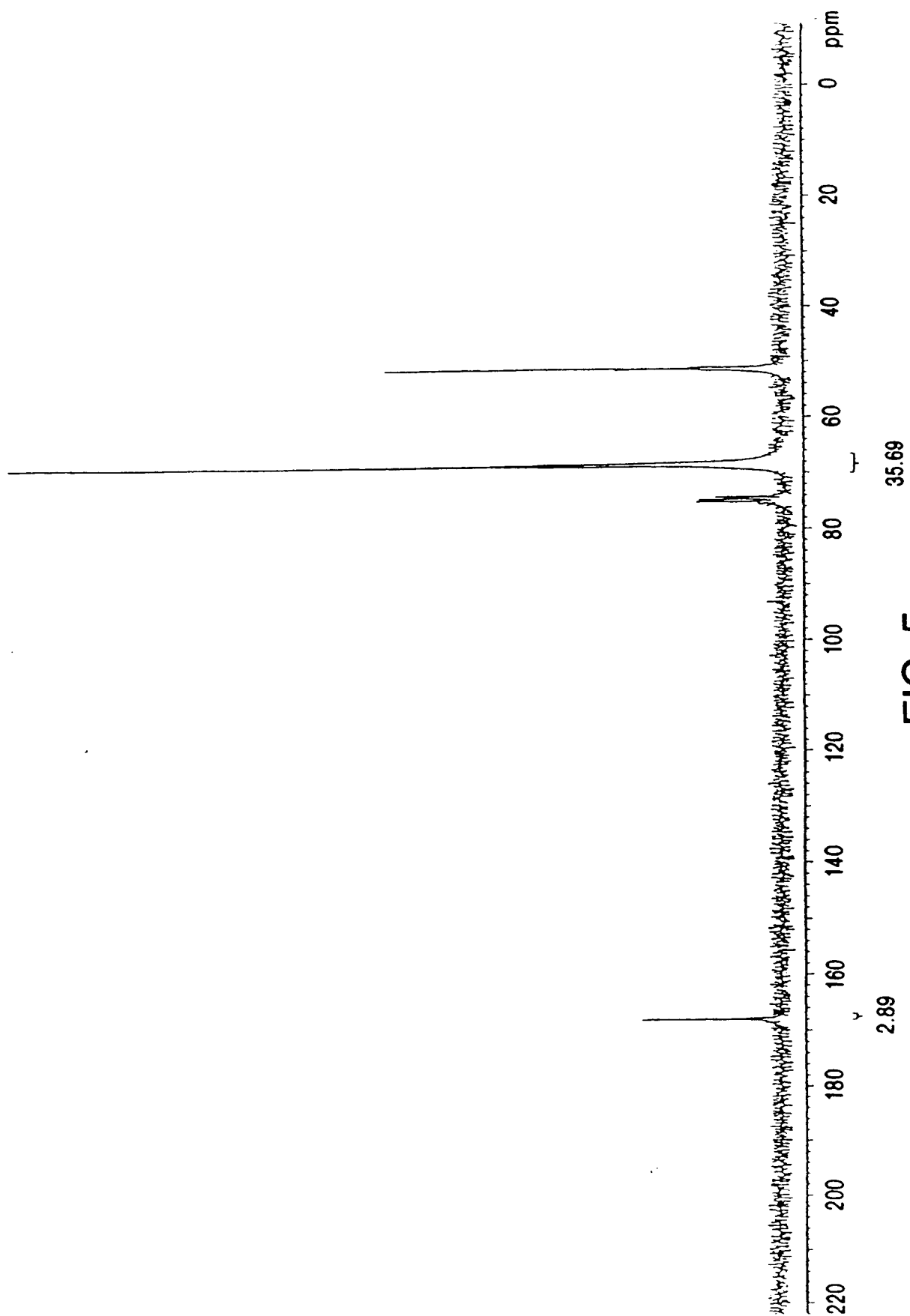


FIG. 5

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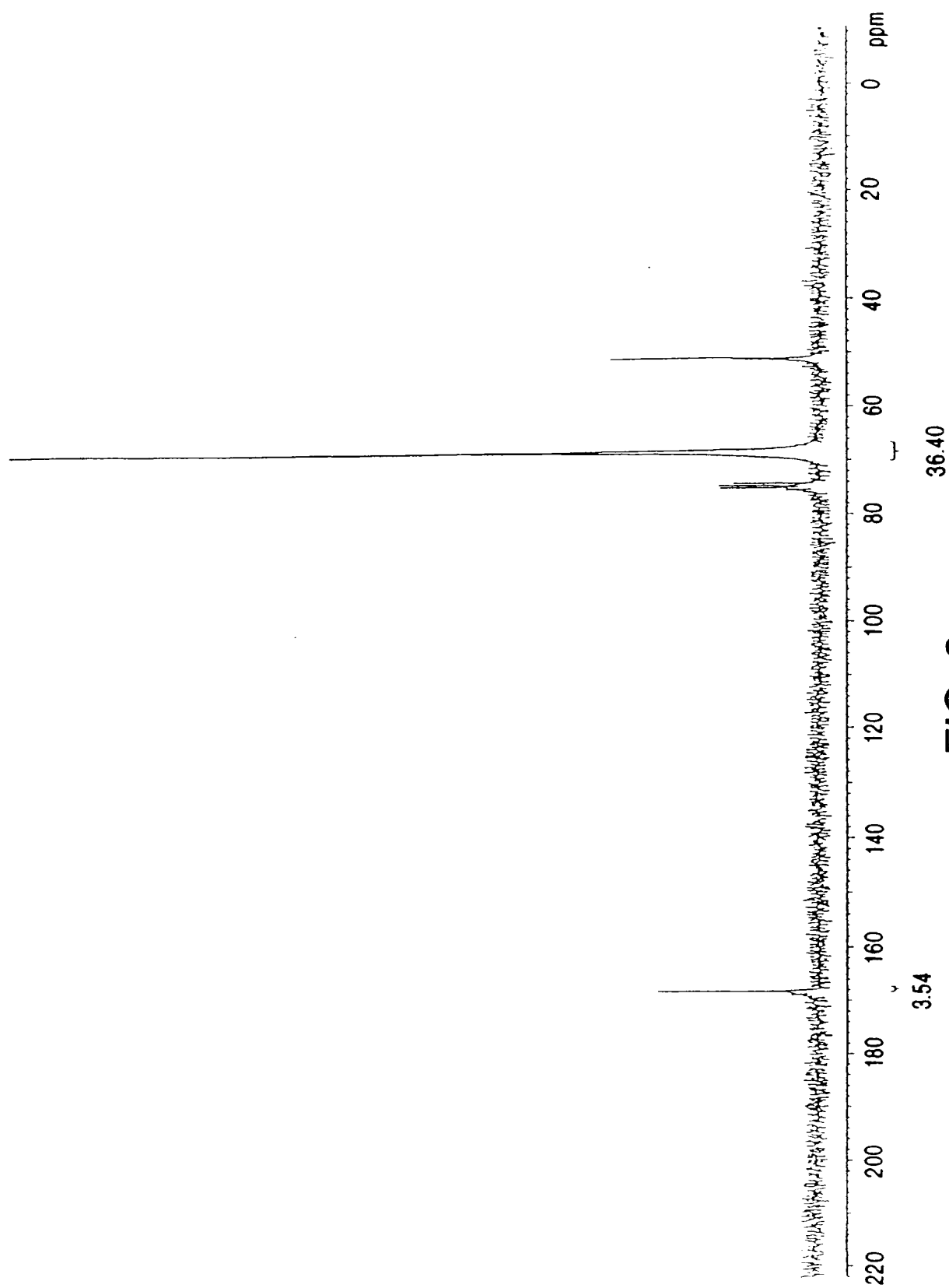


FIG. 6

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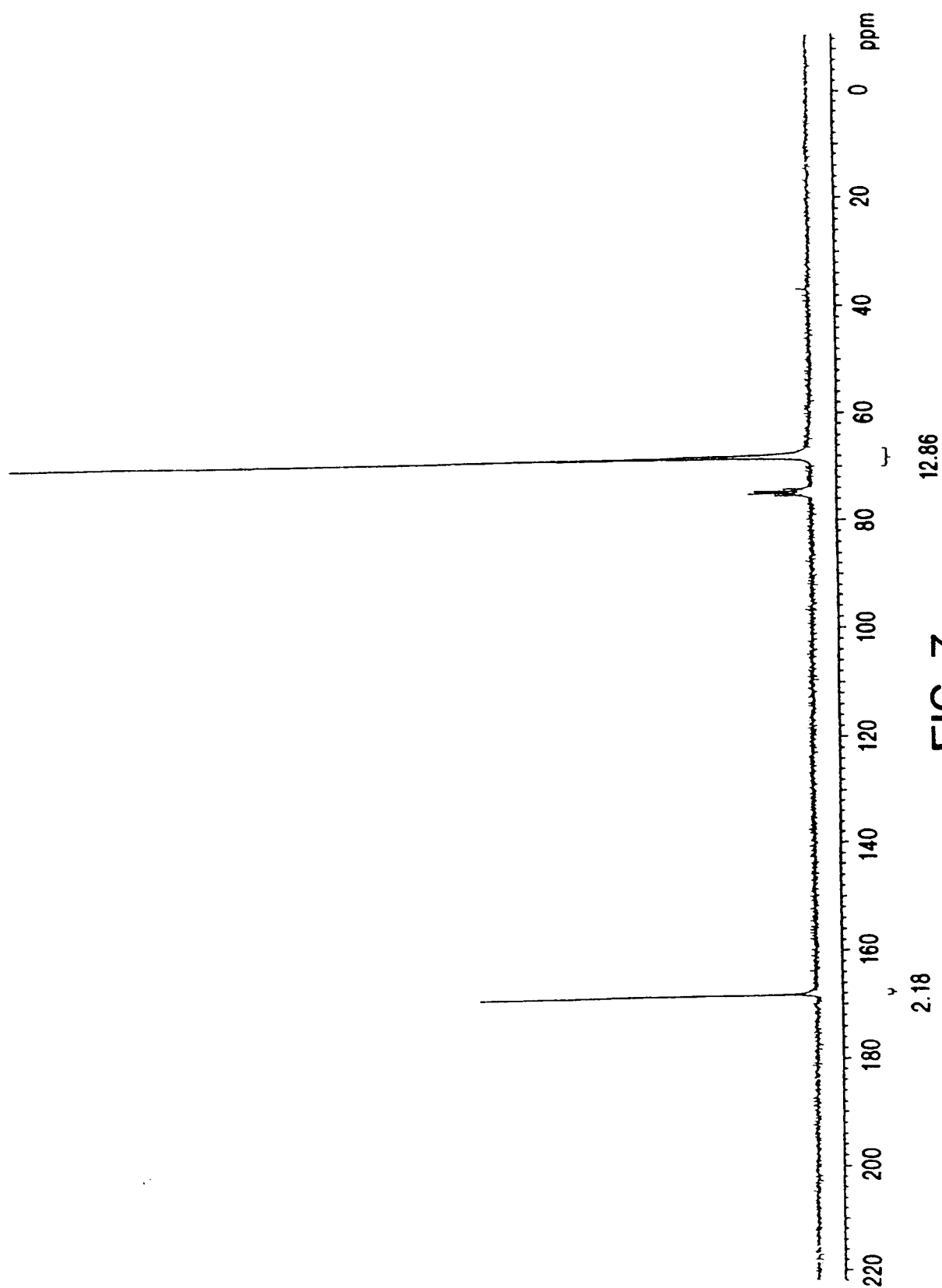


FIG. 7

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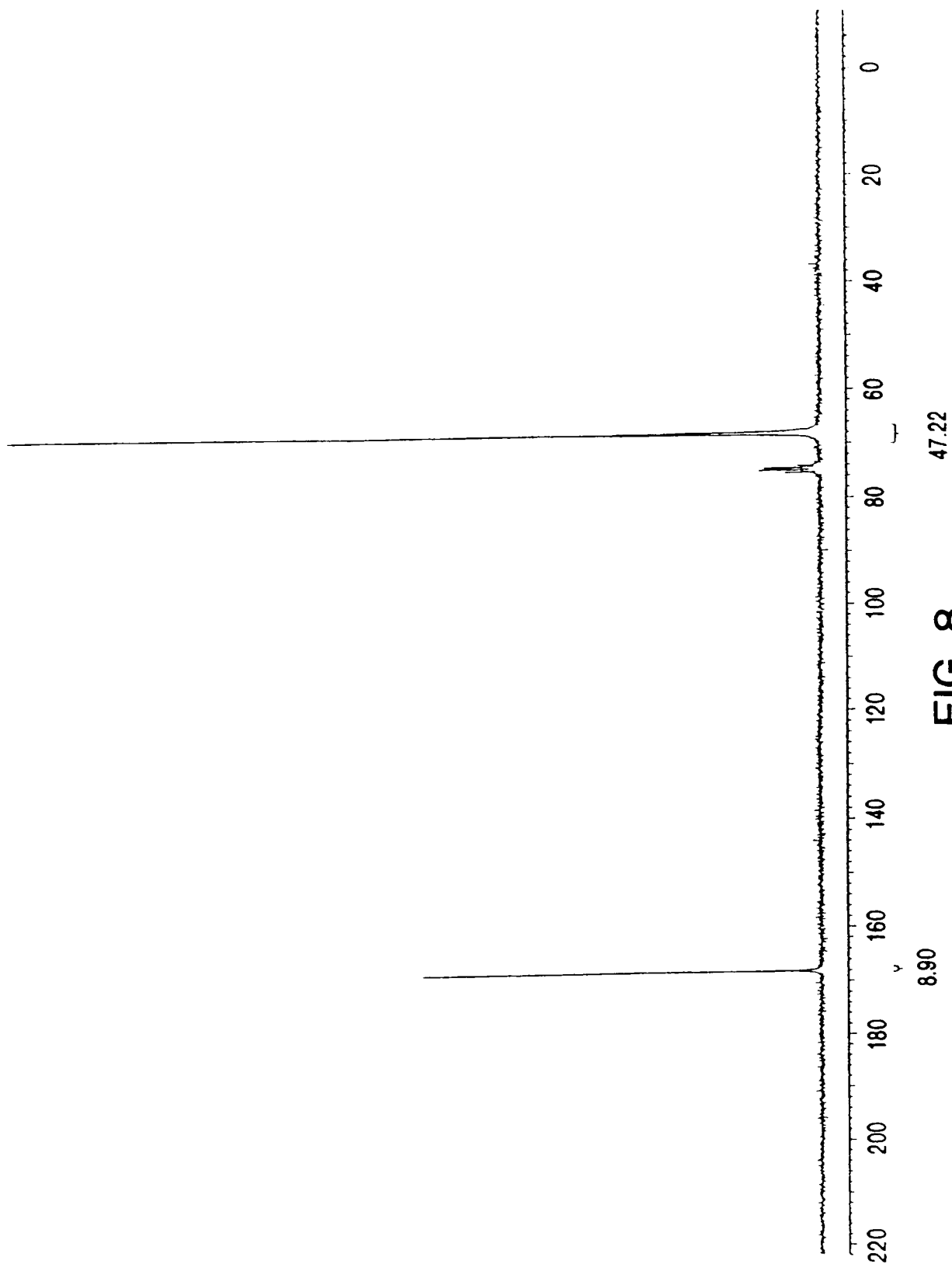


FIG. 8

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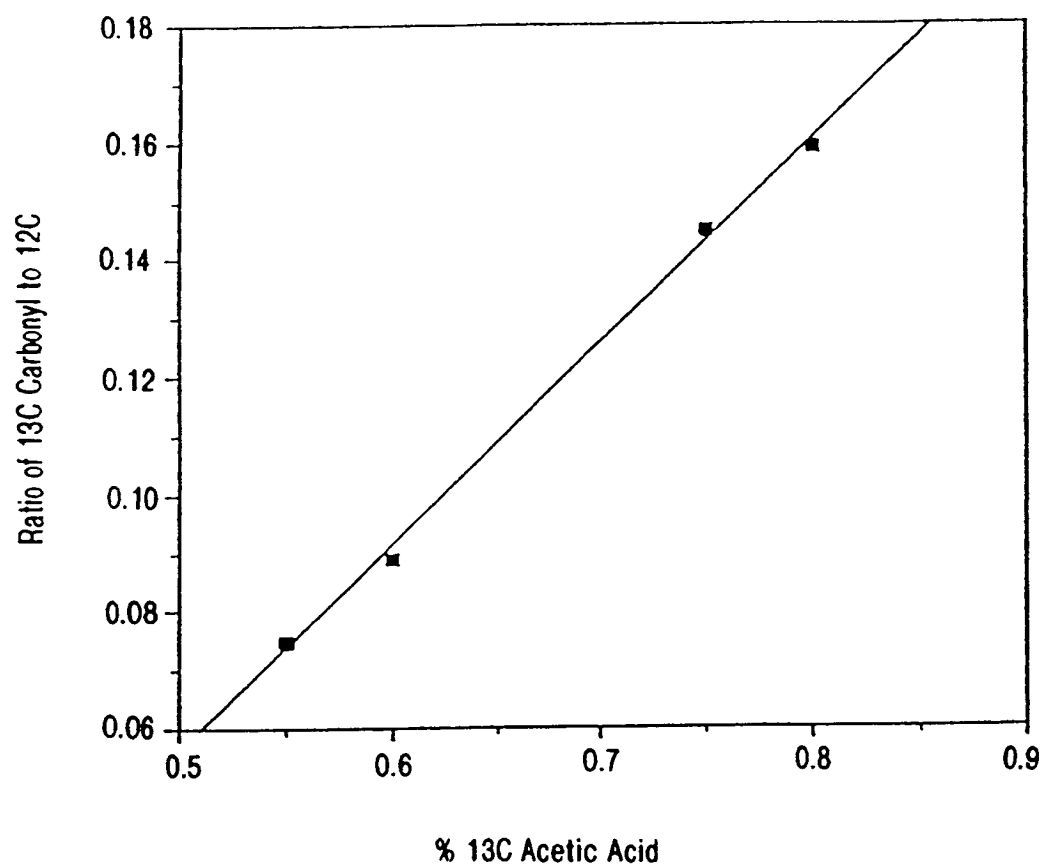
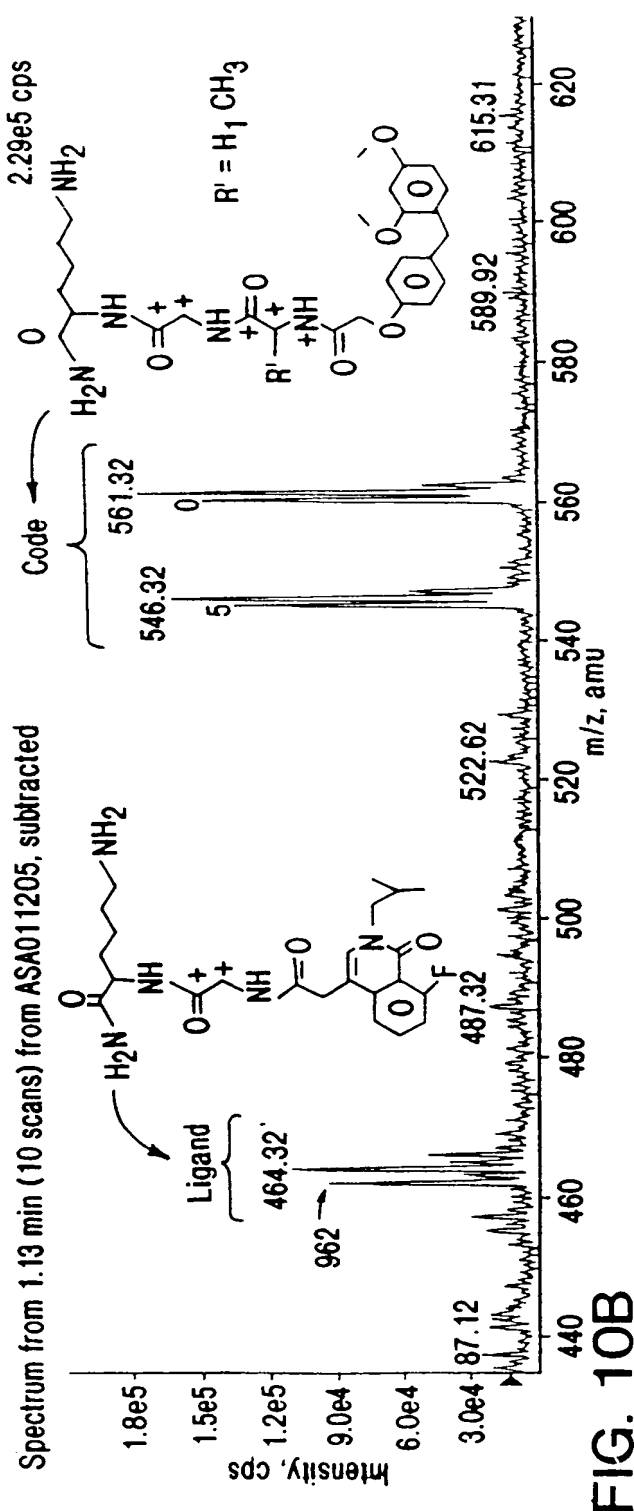
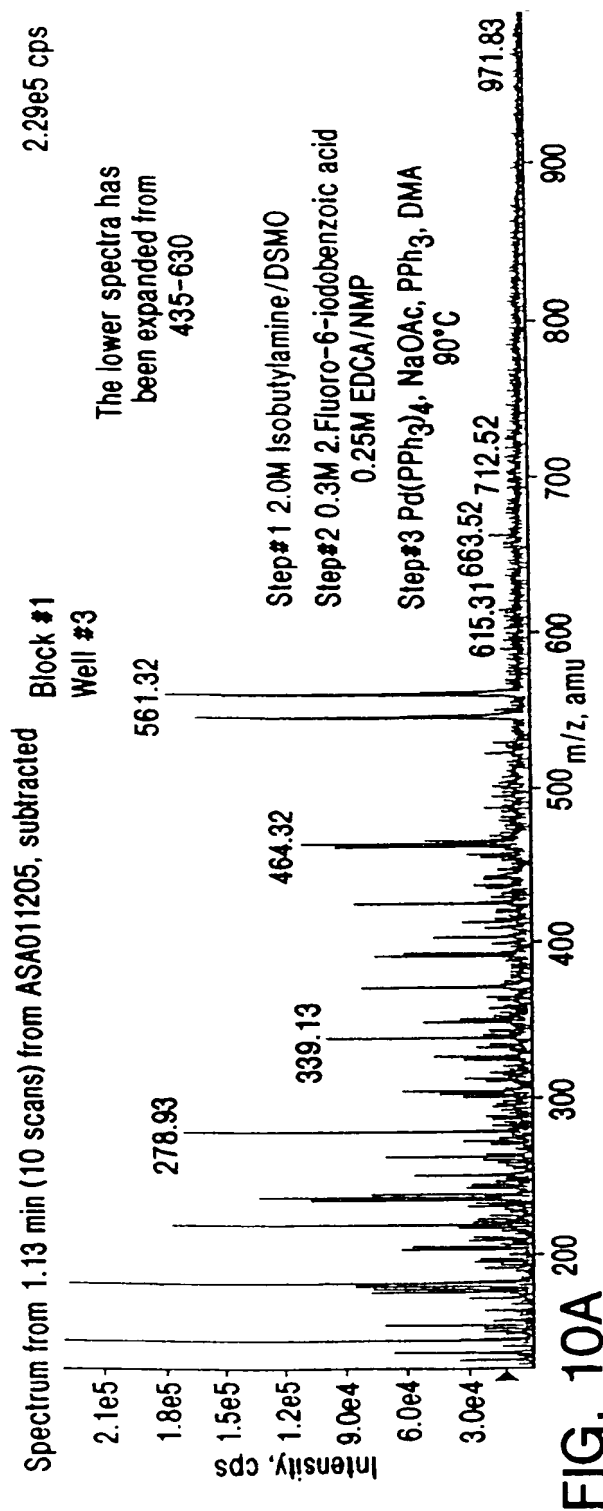
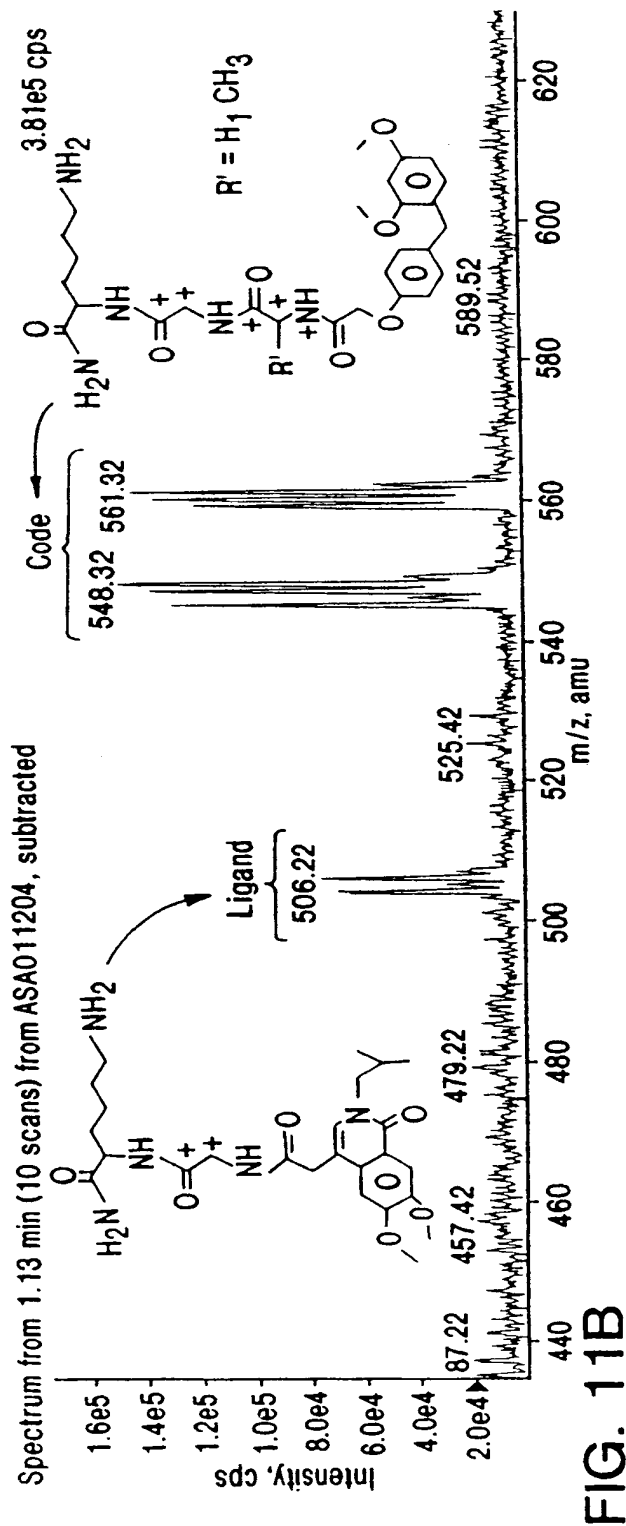
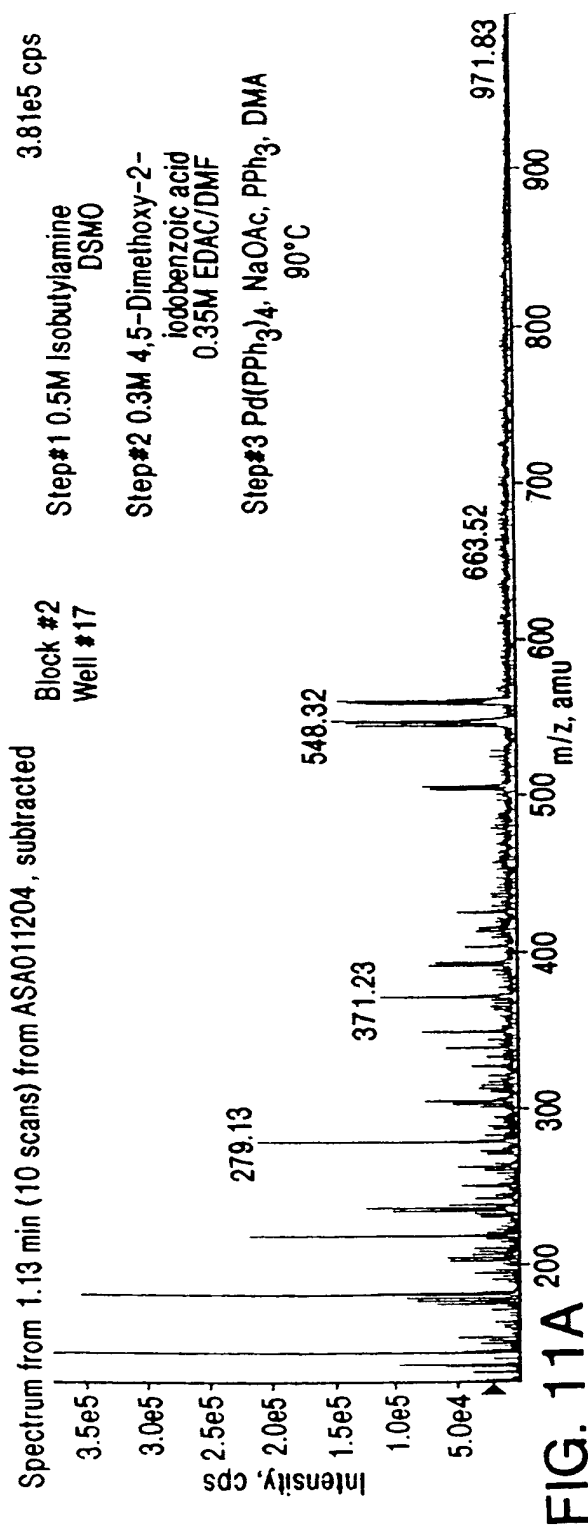


FIG. 9

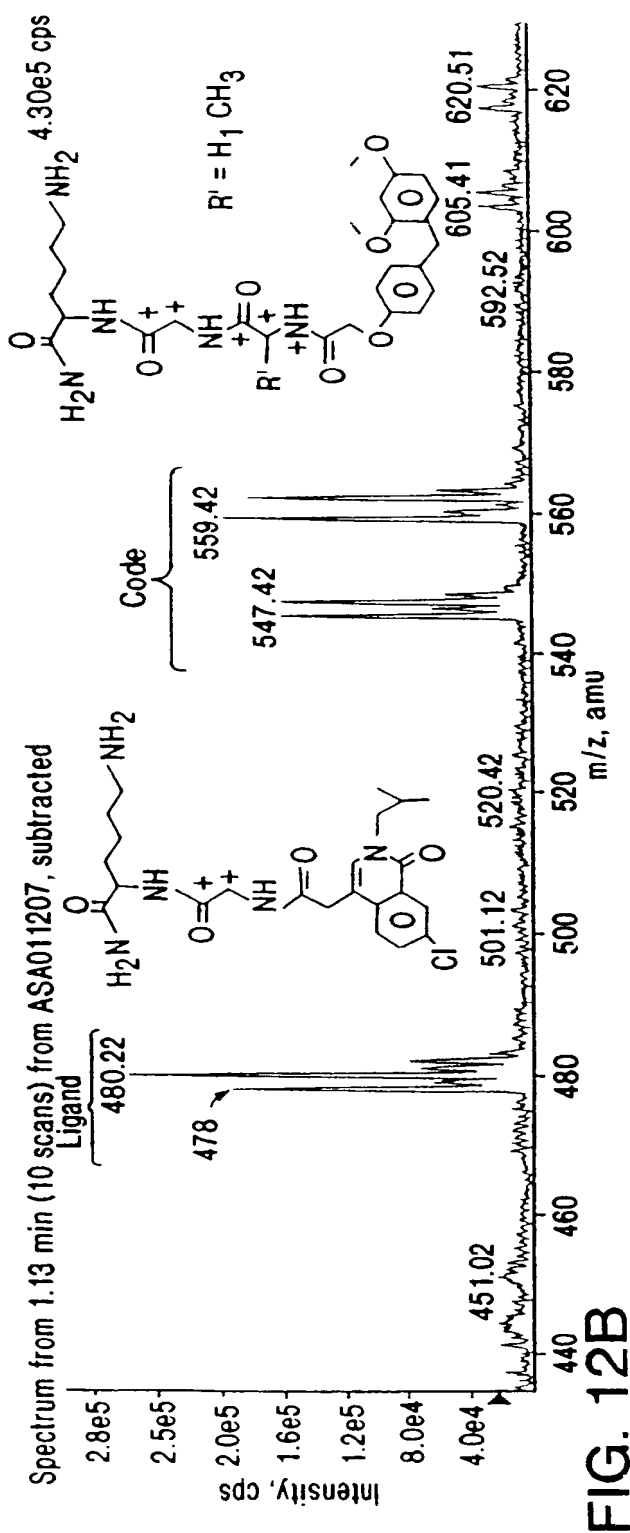
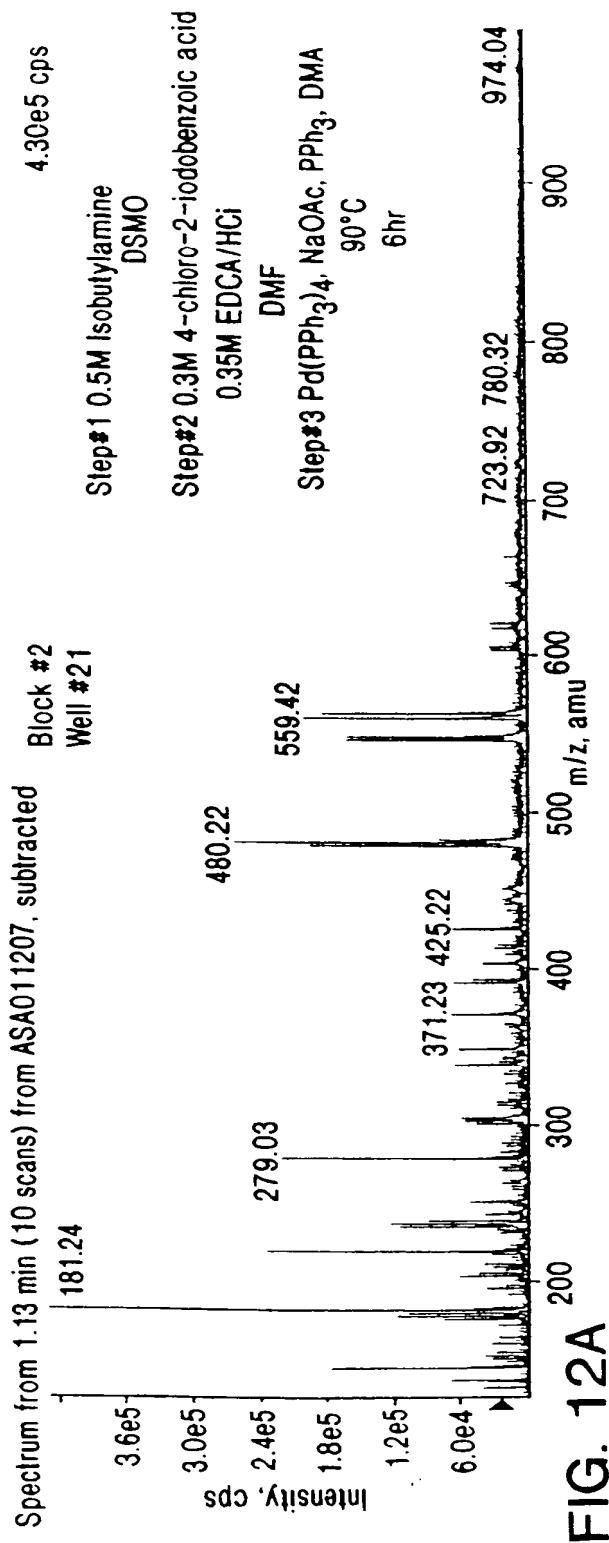
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I. Synthesis of serially encoded tentagel resin.

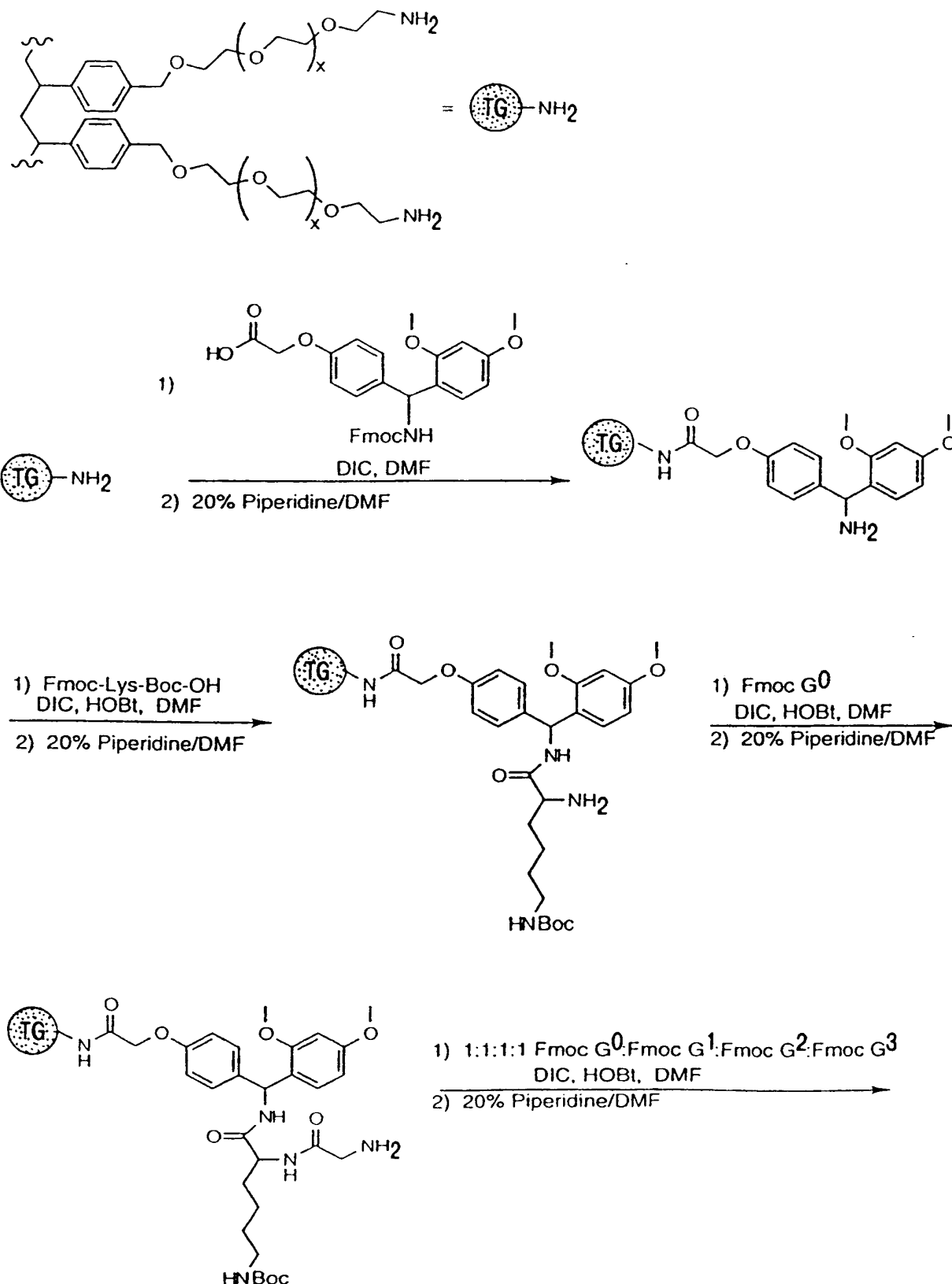


FIG. 13

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I. Synthesis of serially encoded tentagel resin.

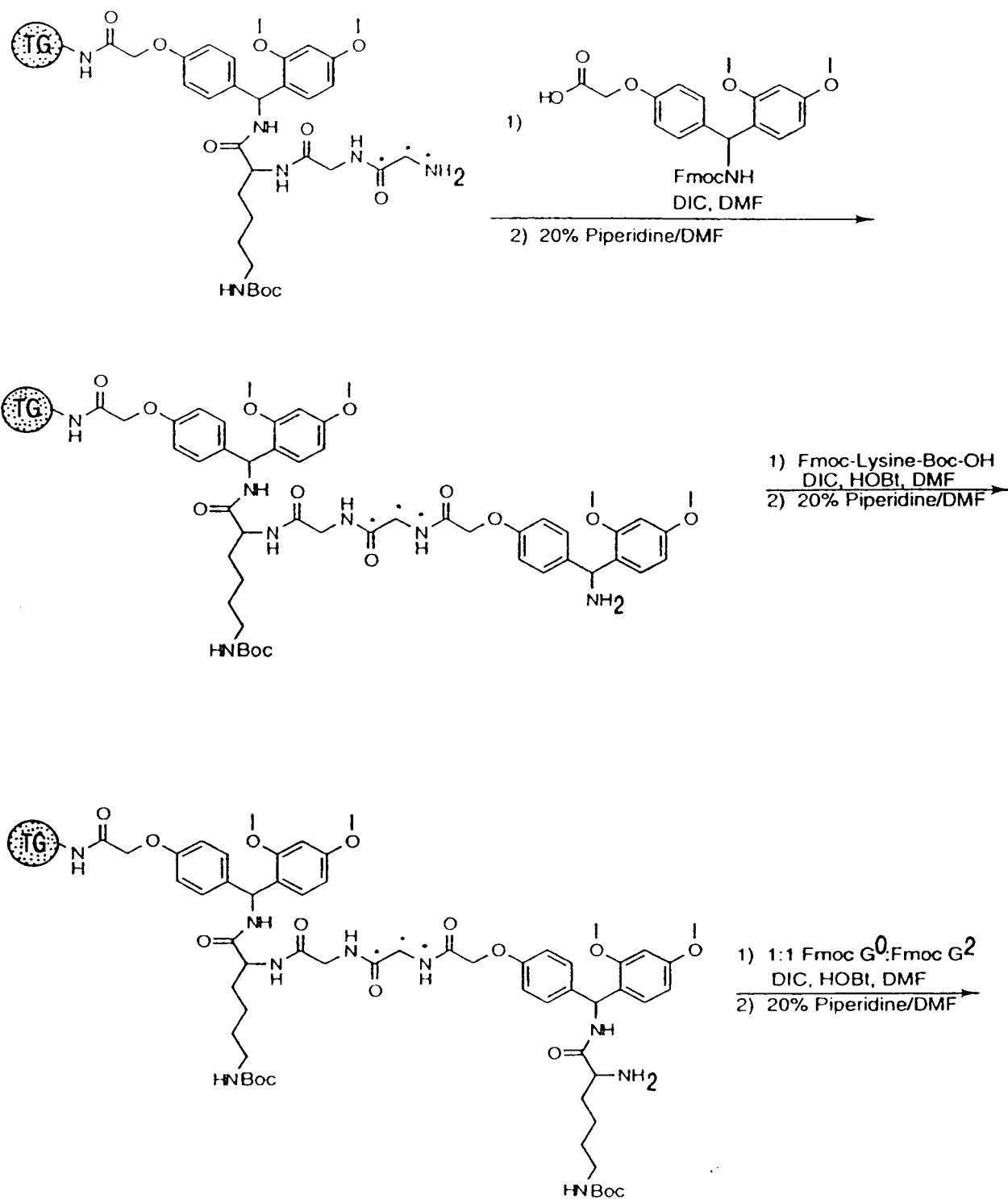


FIG. 14

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II. Reaction Screening of 1(2H)-Isoquinolinones with serially encoded tentagel resin.

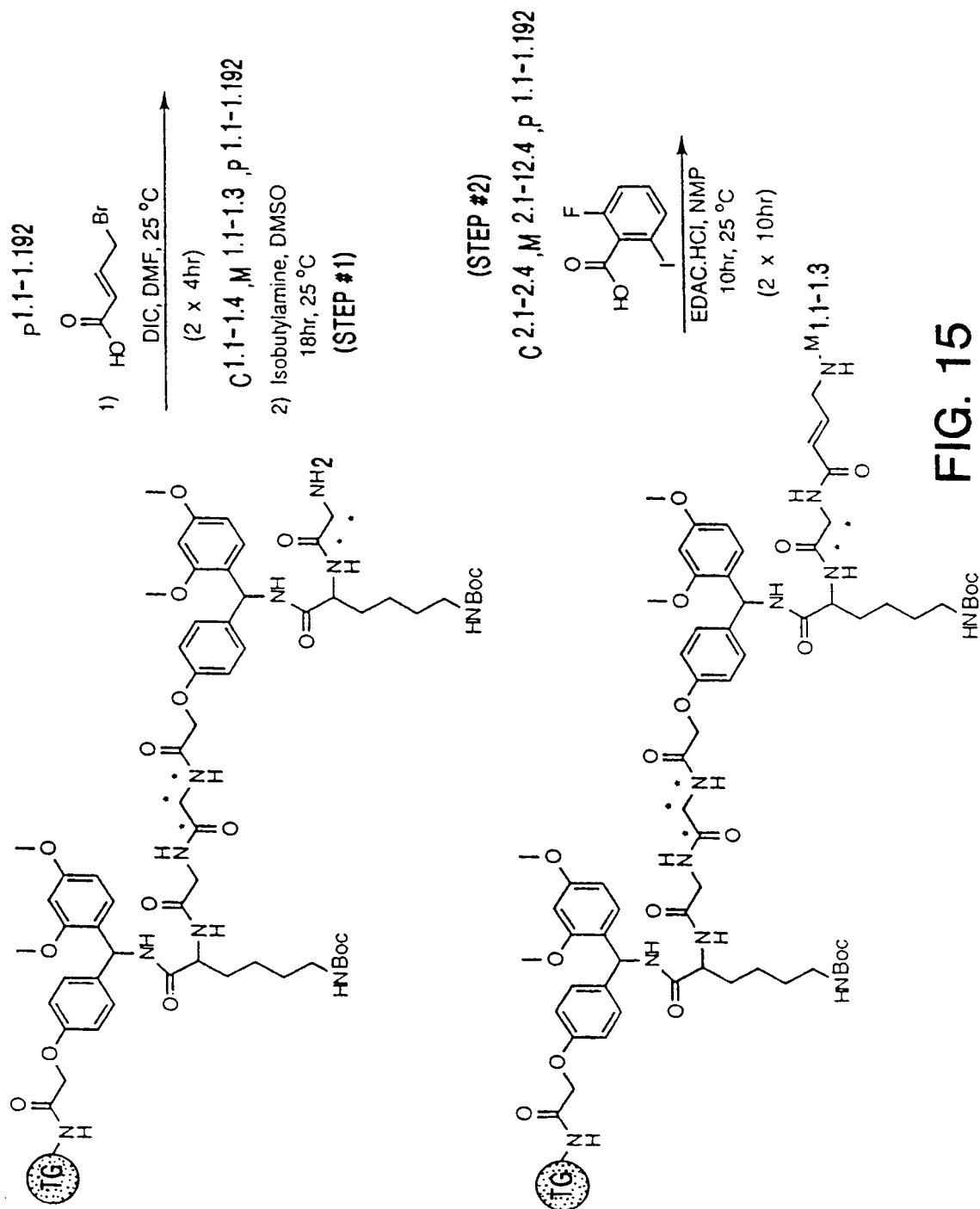


FIG. 15

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II. Reaction Screening of 1(2H)-Isoquinolinones with serially encoded tentagel resin. (STEP #3)

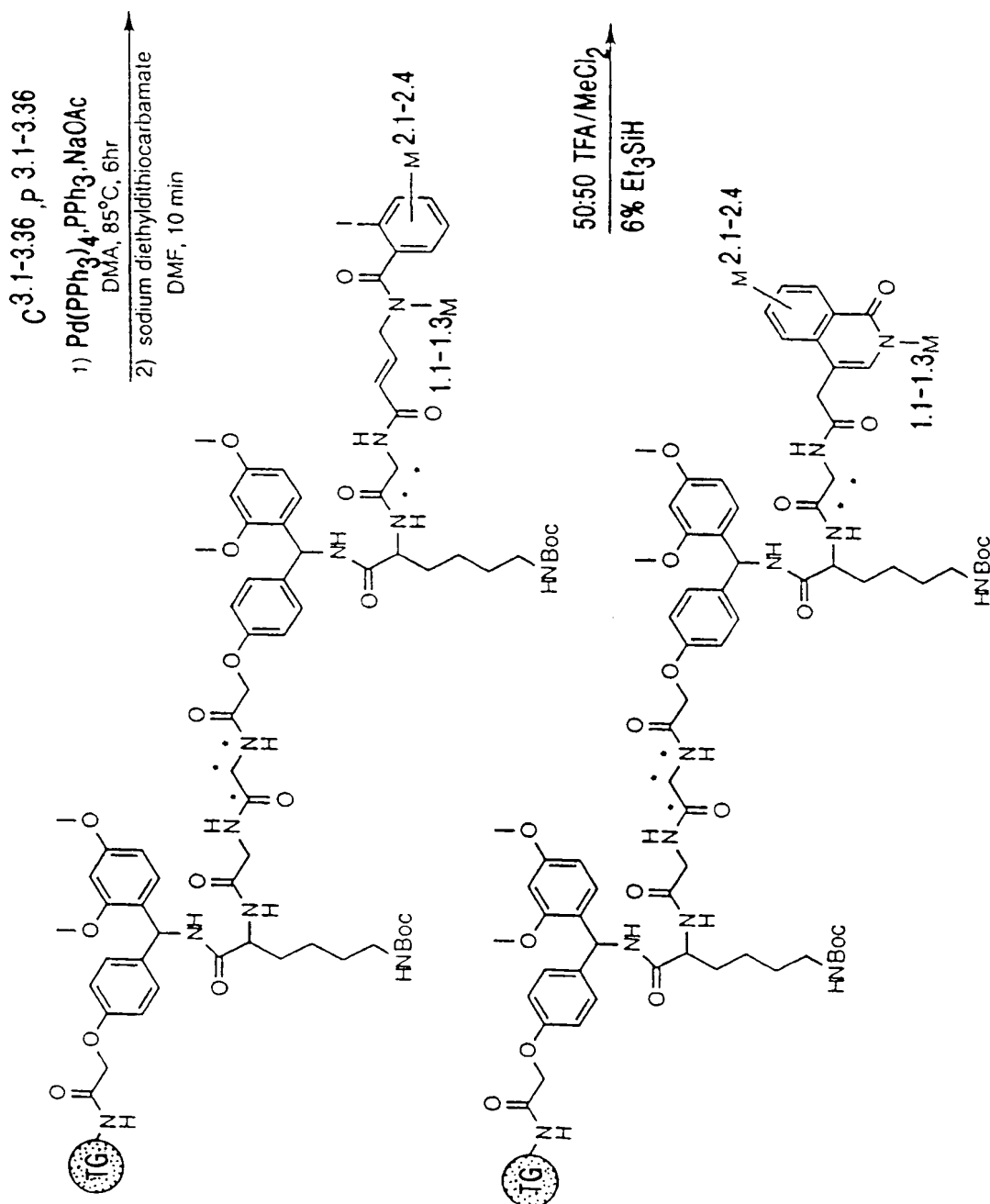


FIG. 16

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II. Reaction Screening of 1(2H)-Isoquinolinones with serially encoded tentagel resin.

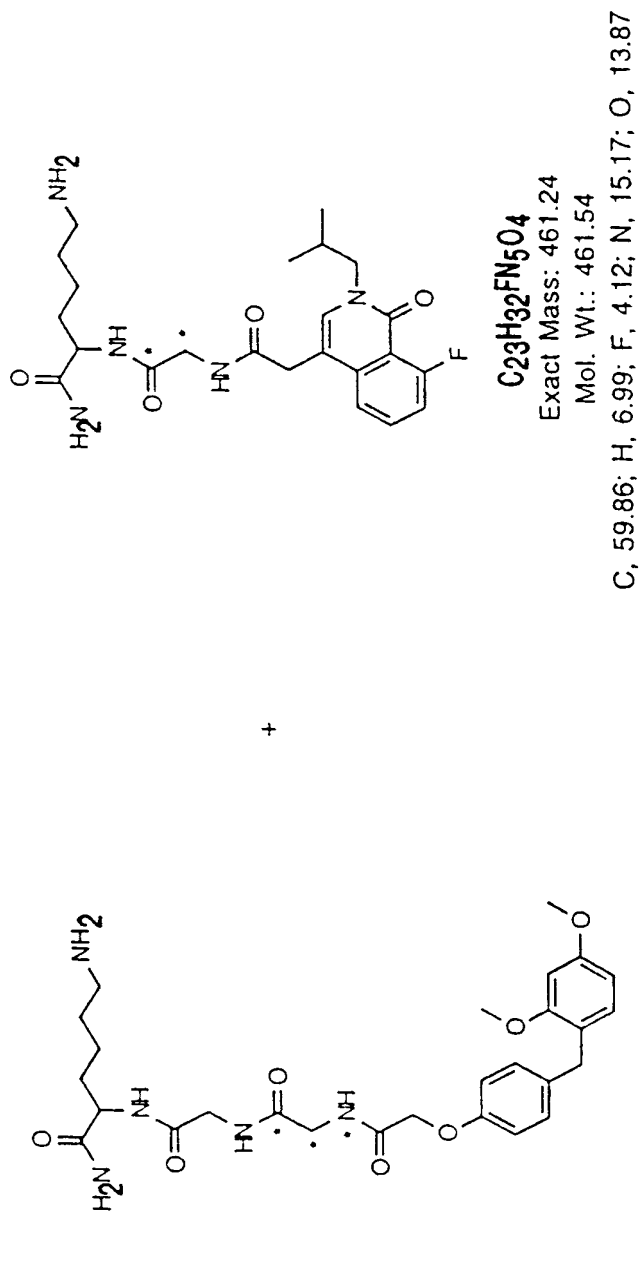


FIG. 17

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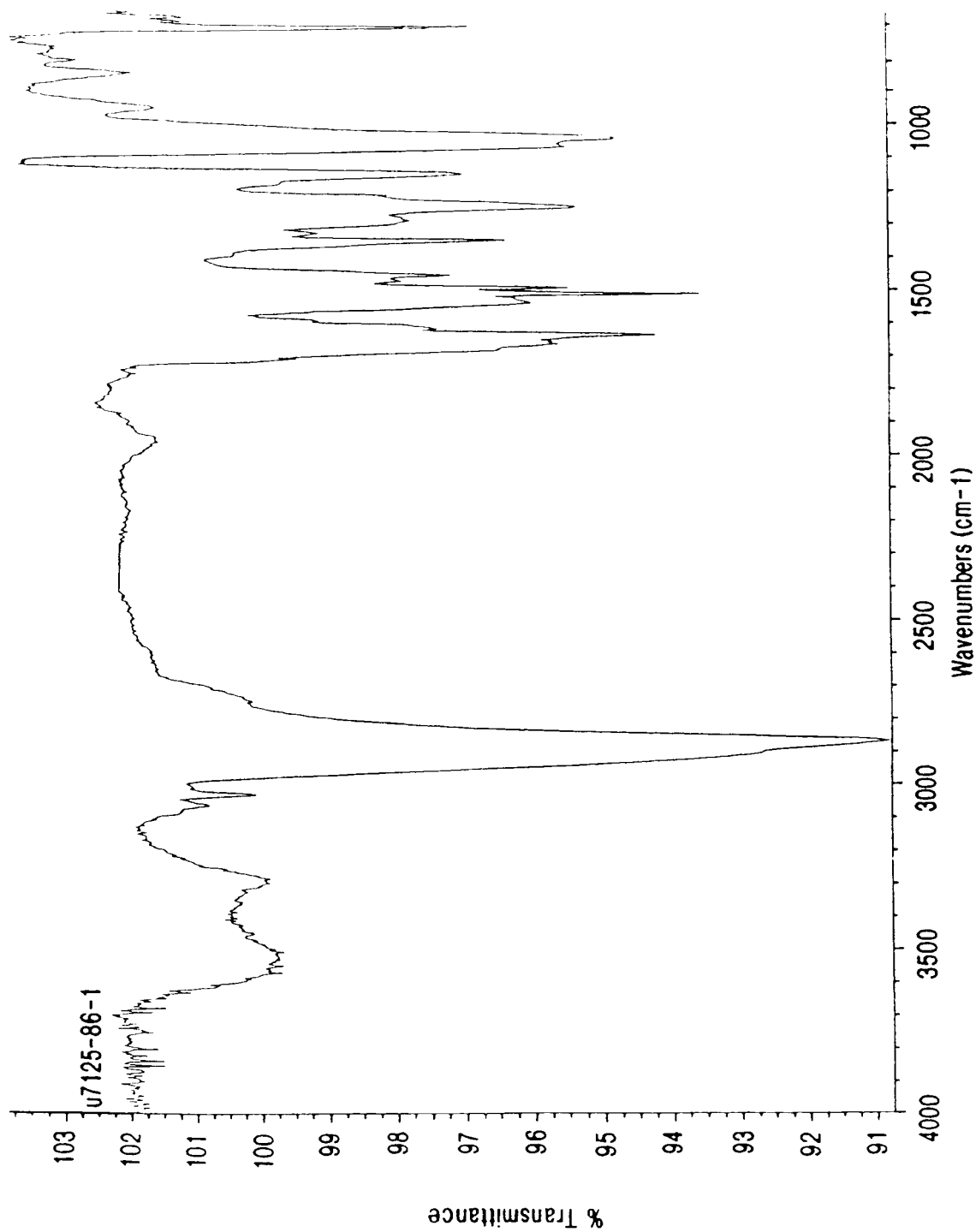


FIG. 18

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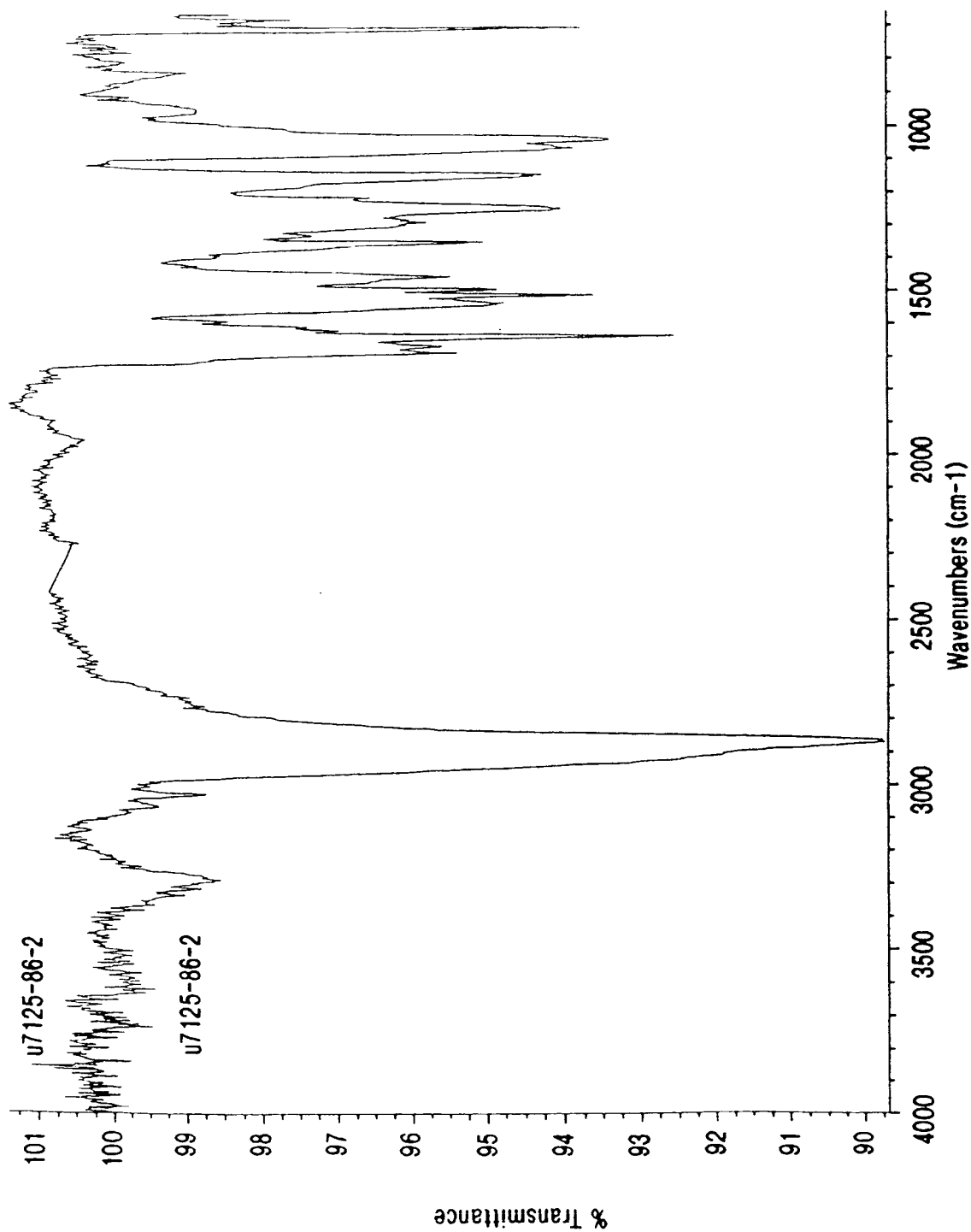


FIG. 19

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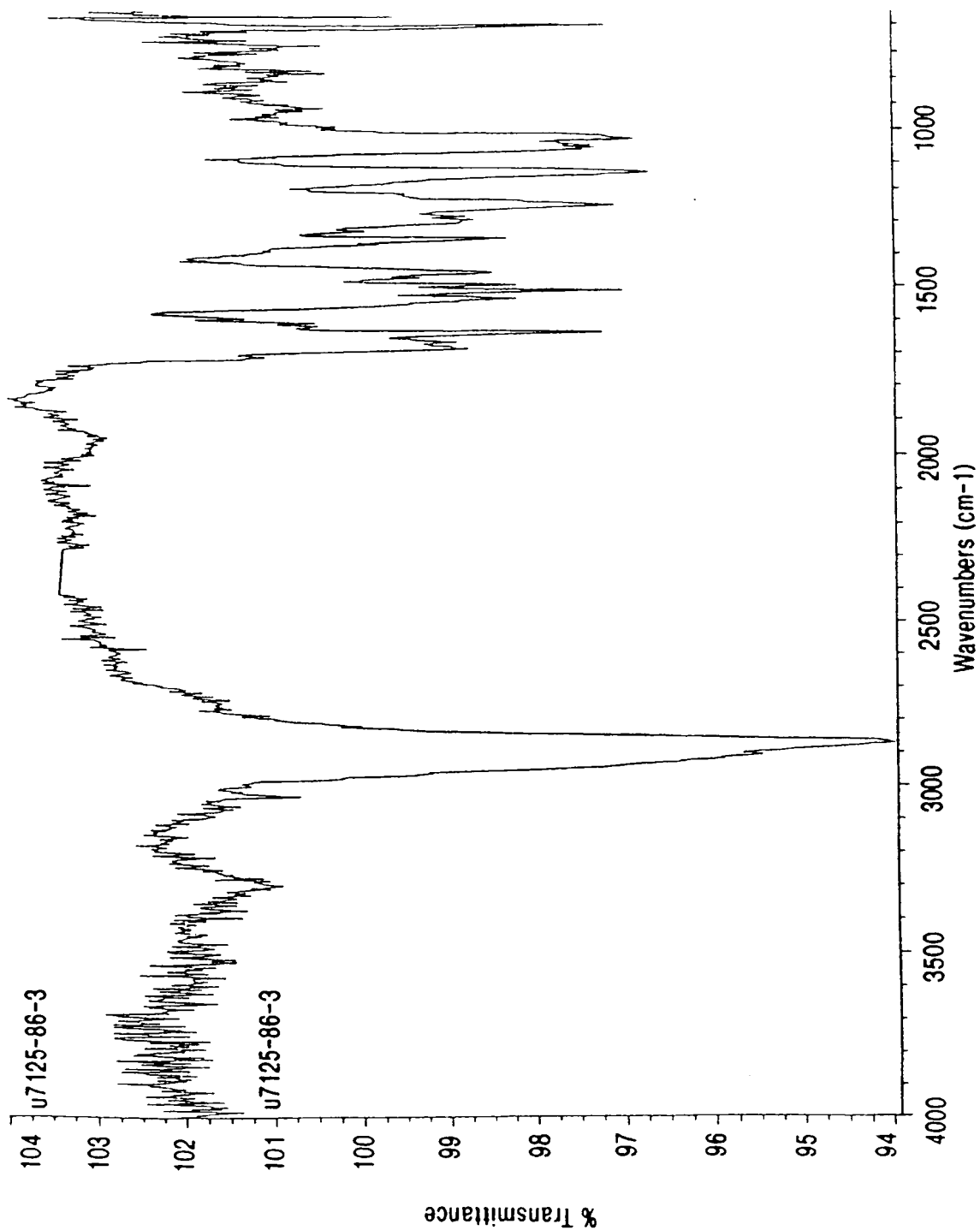


FIG. 20

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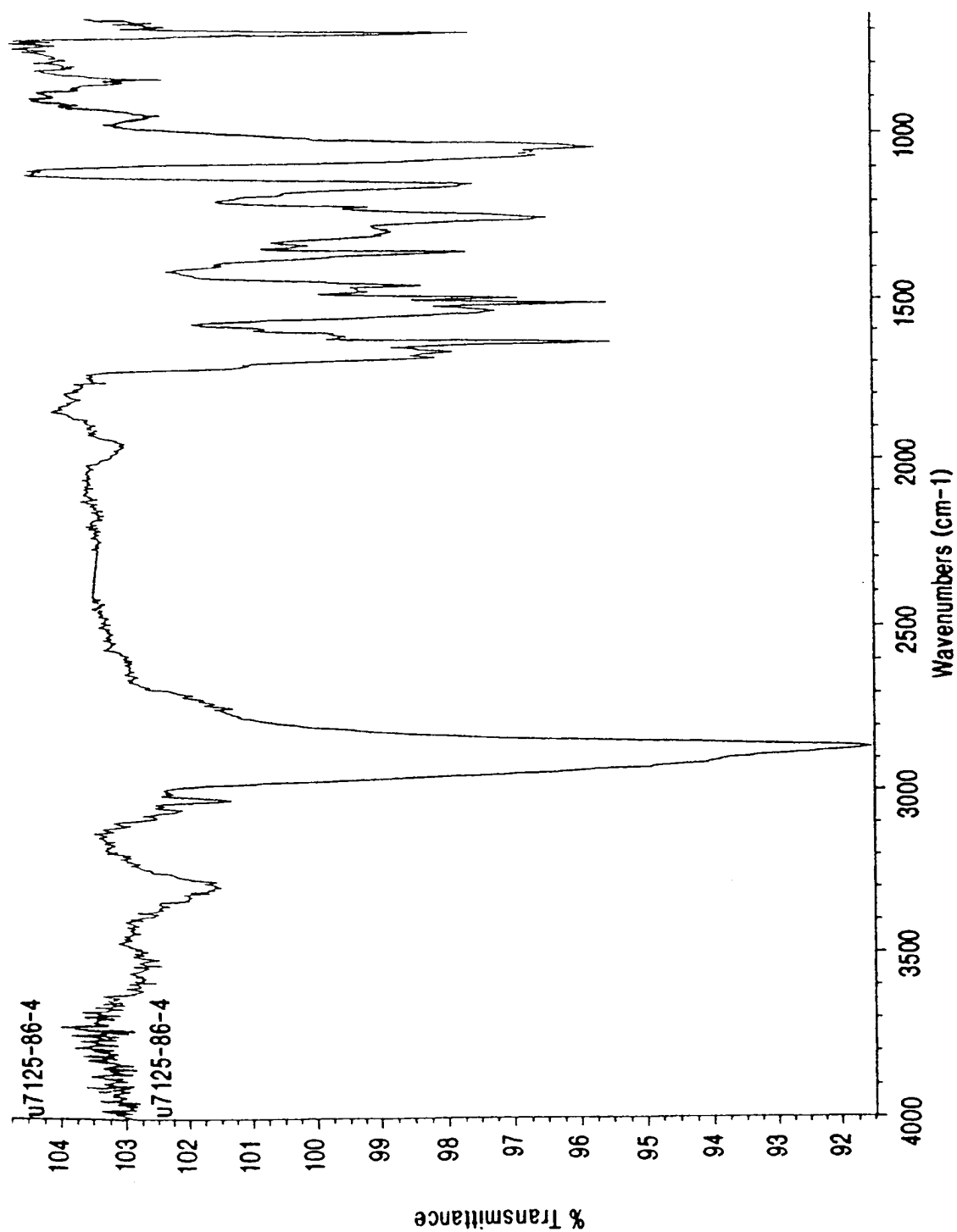


FIG. 21

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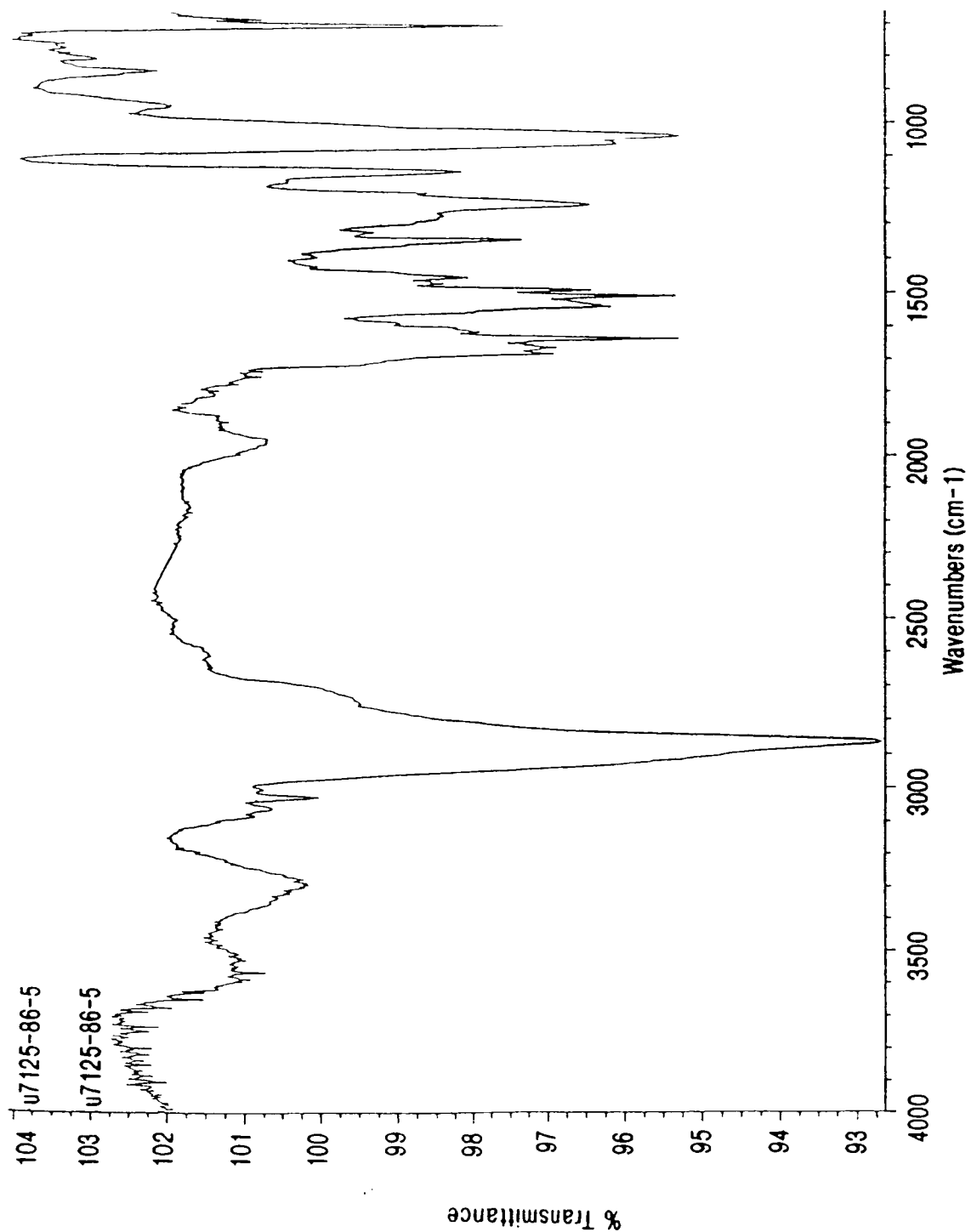


FIG. 22

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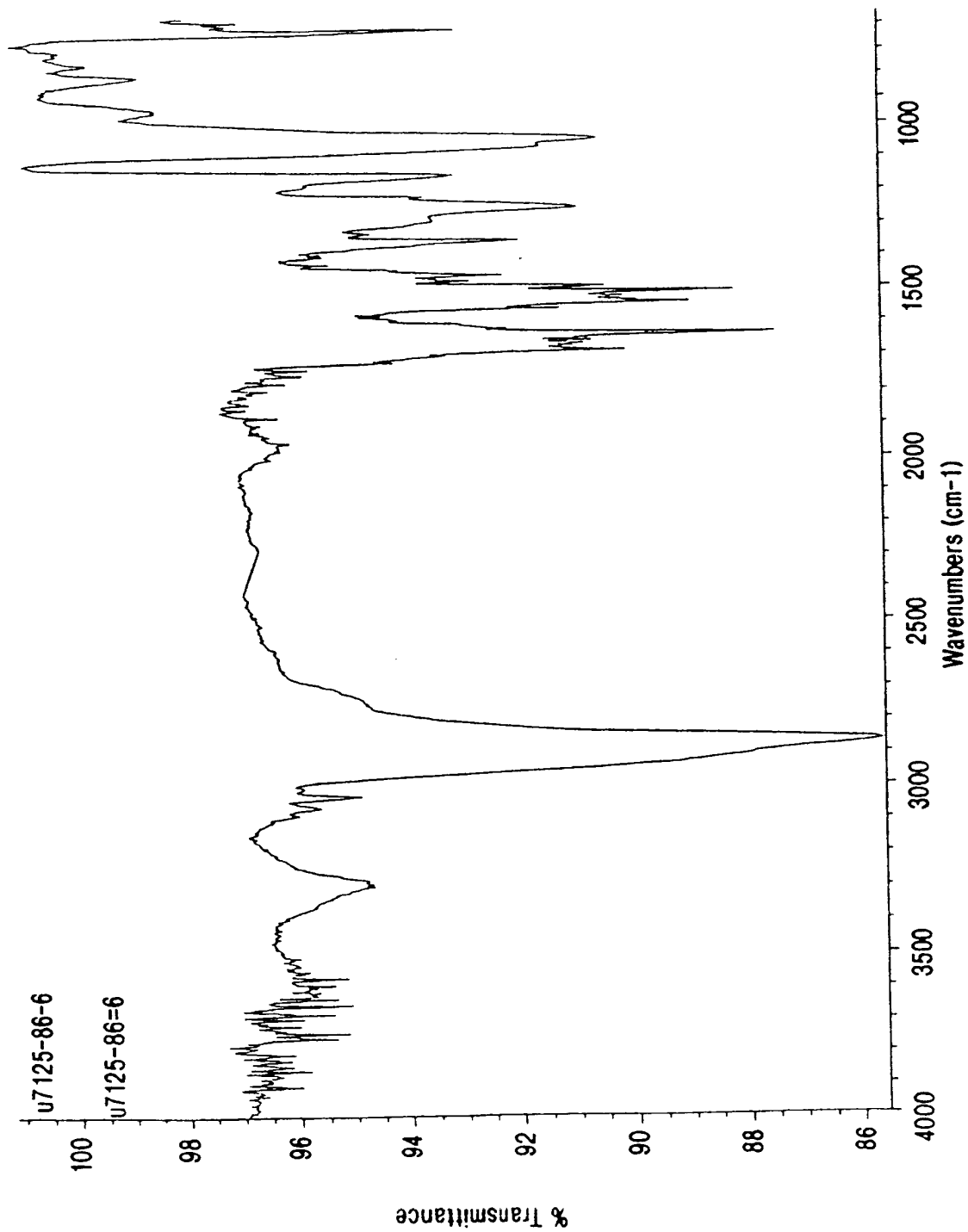


FIG. 23

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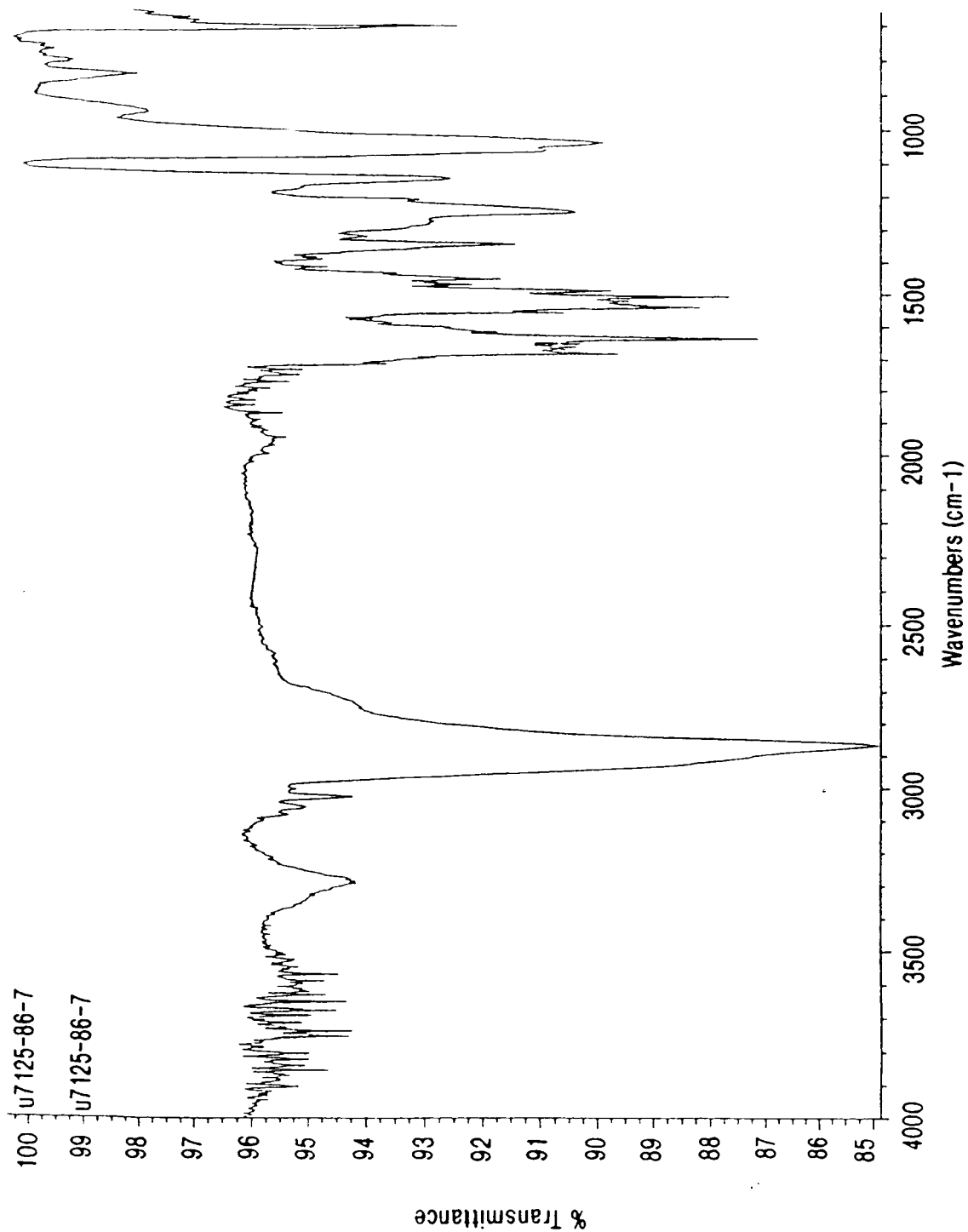


FIG. 24

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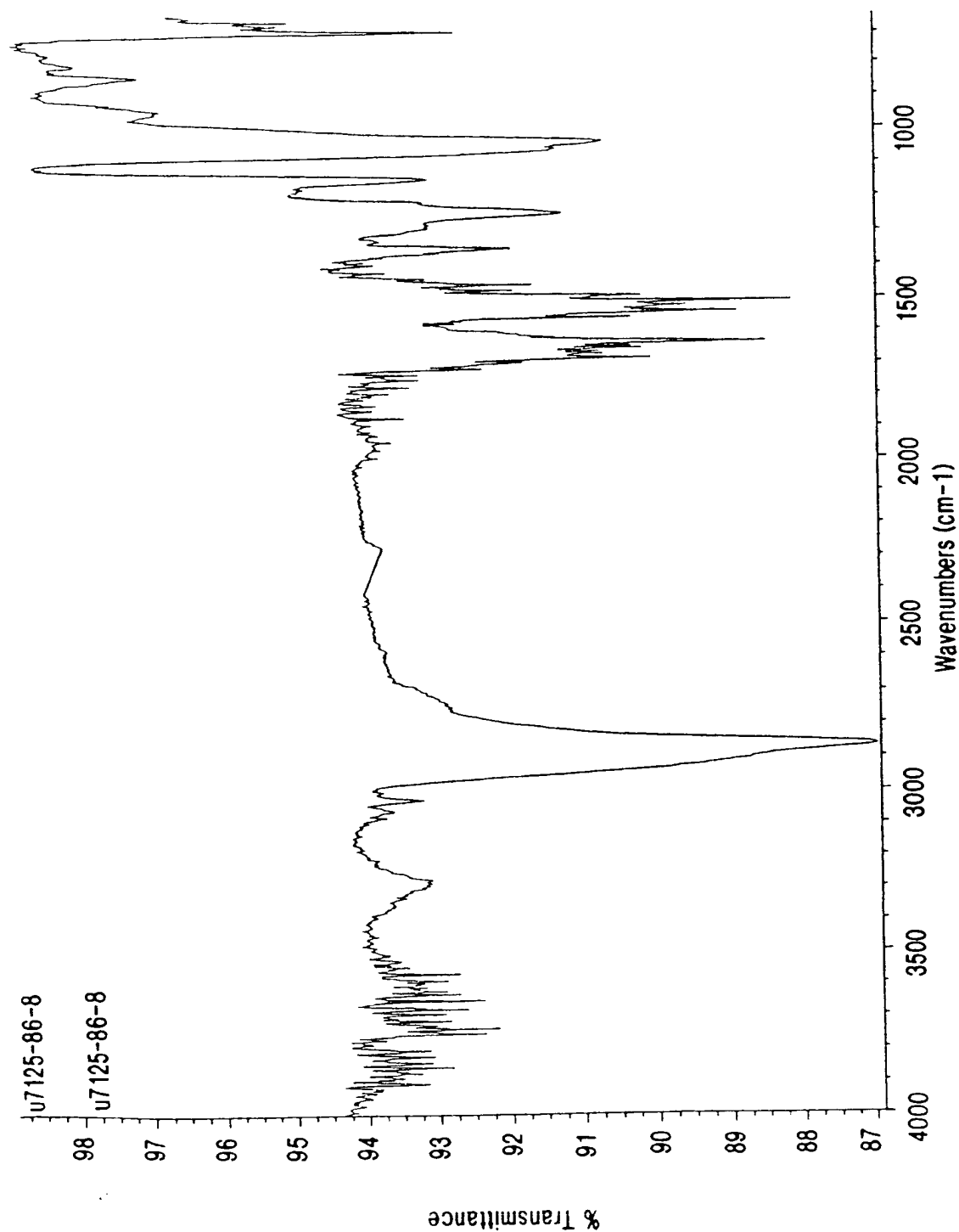


FIG. 25

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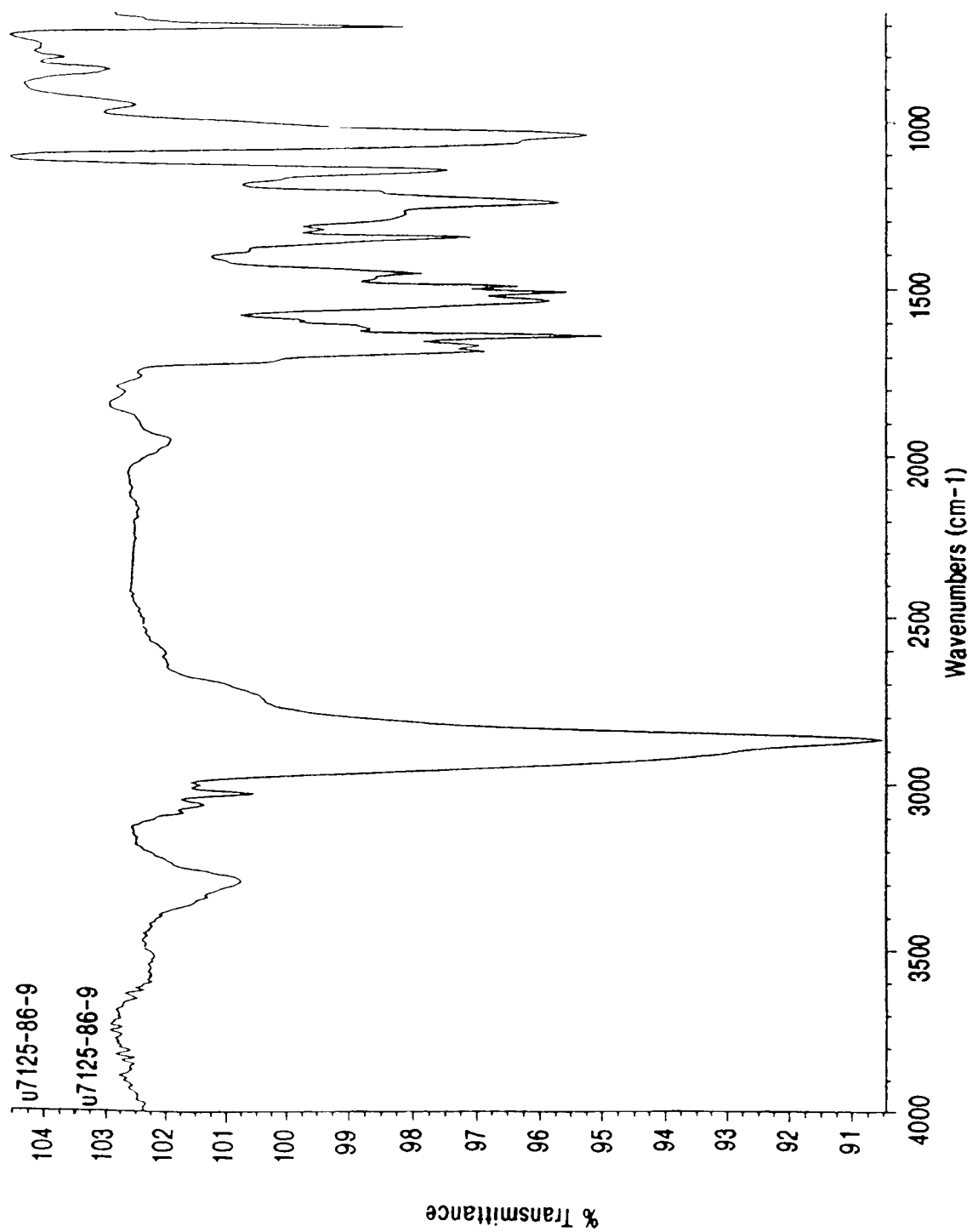


FIG. 26

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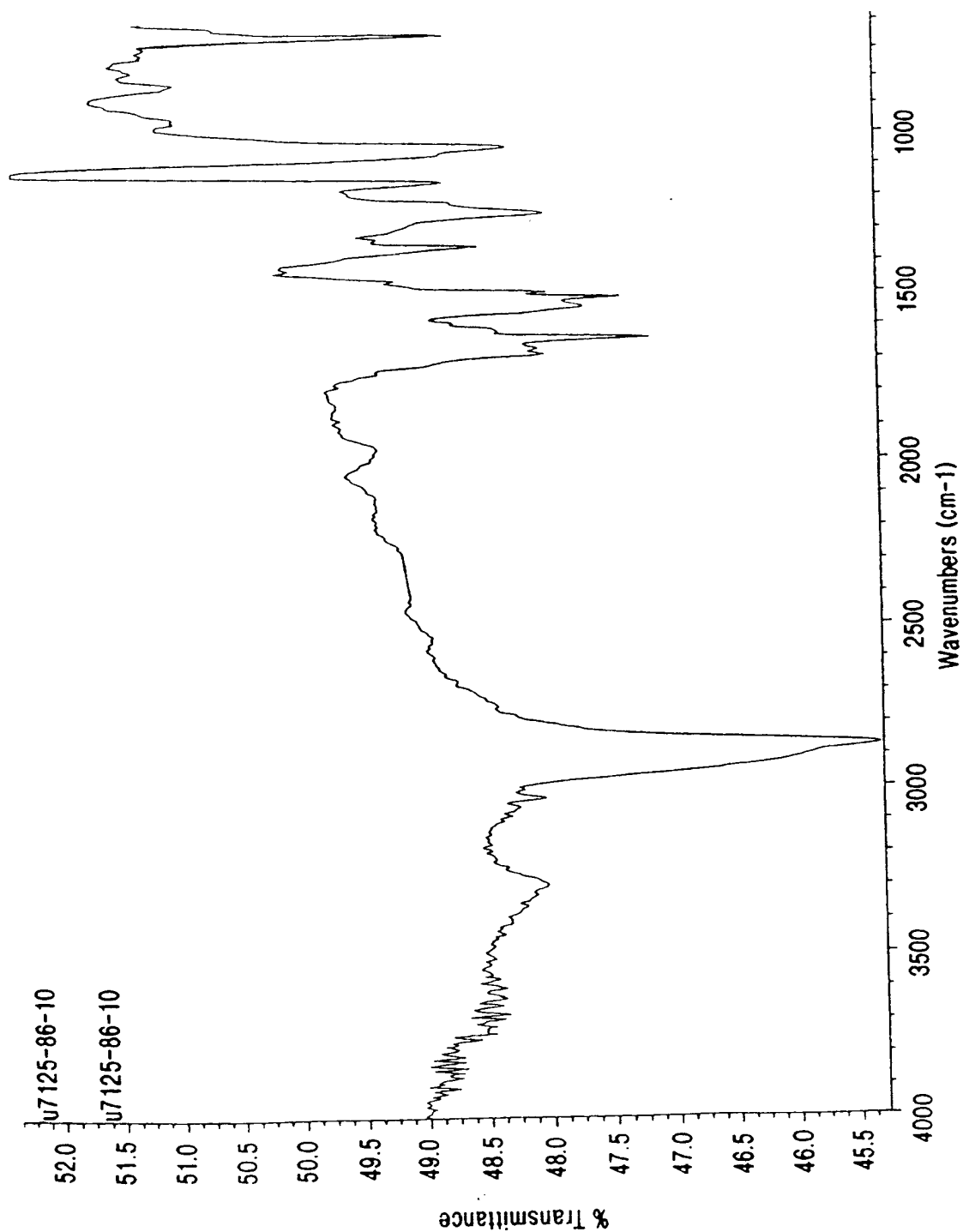


FIG. 27

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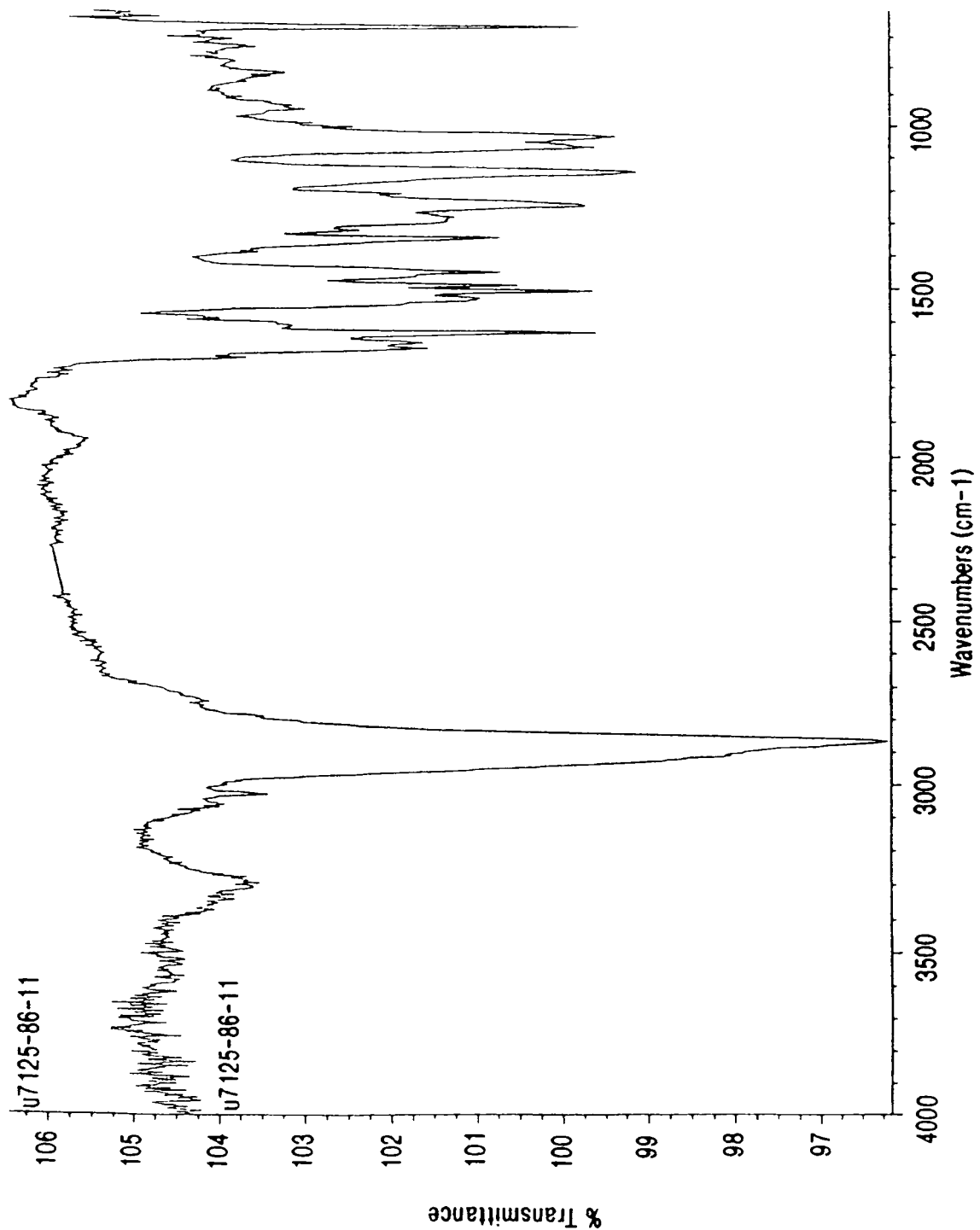


FIG. 28

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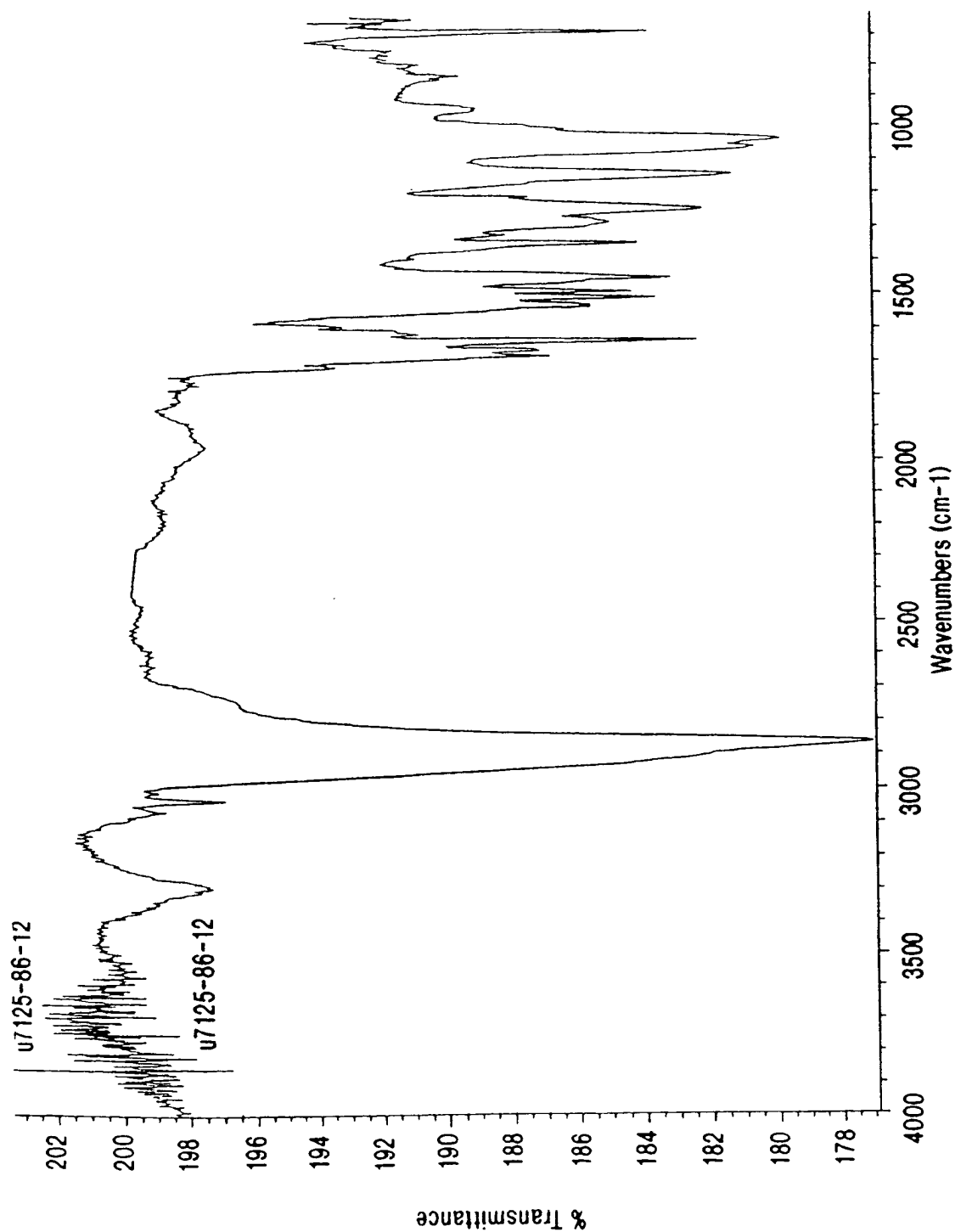


FIG. 29

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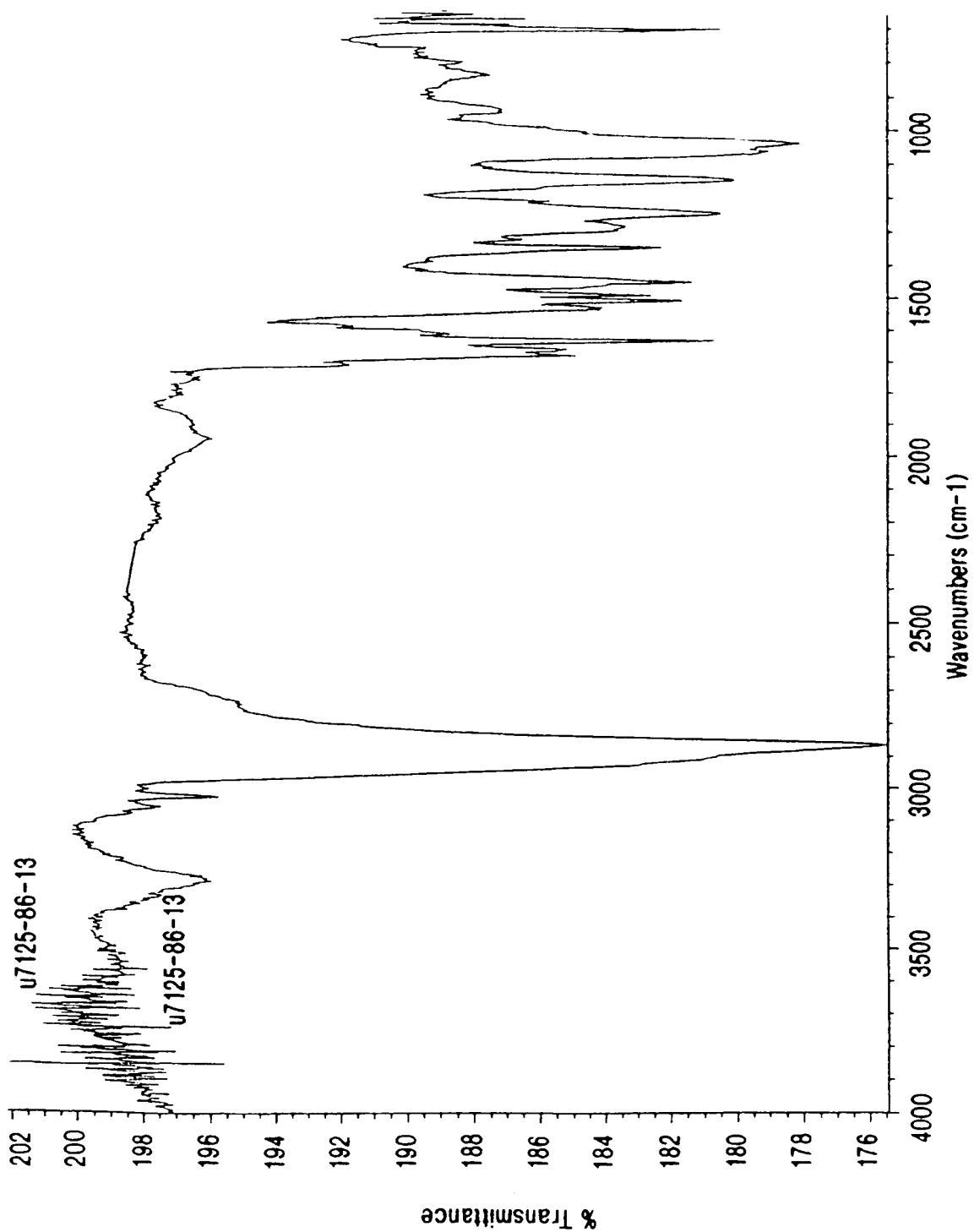


FIG. 30

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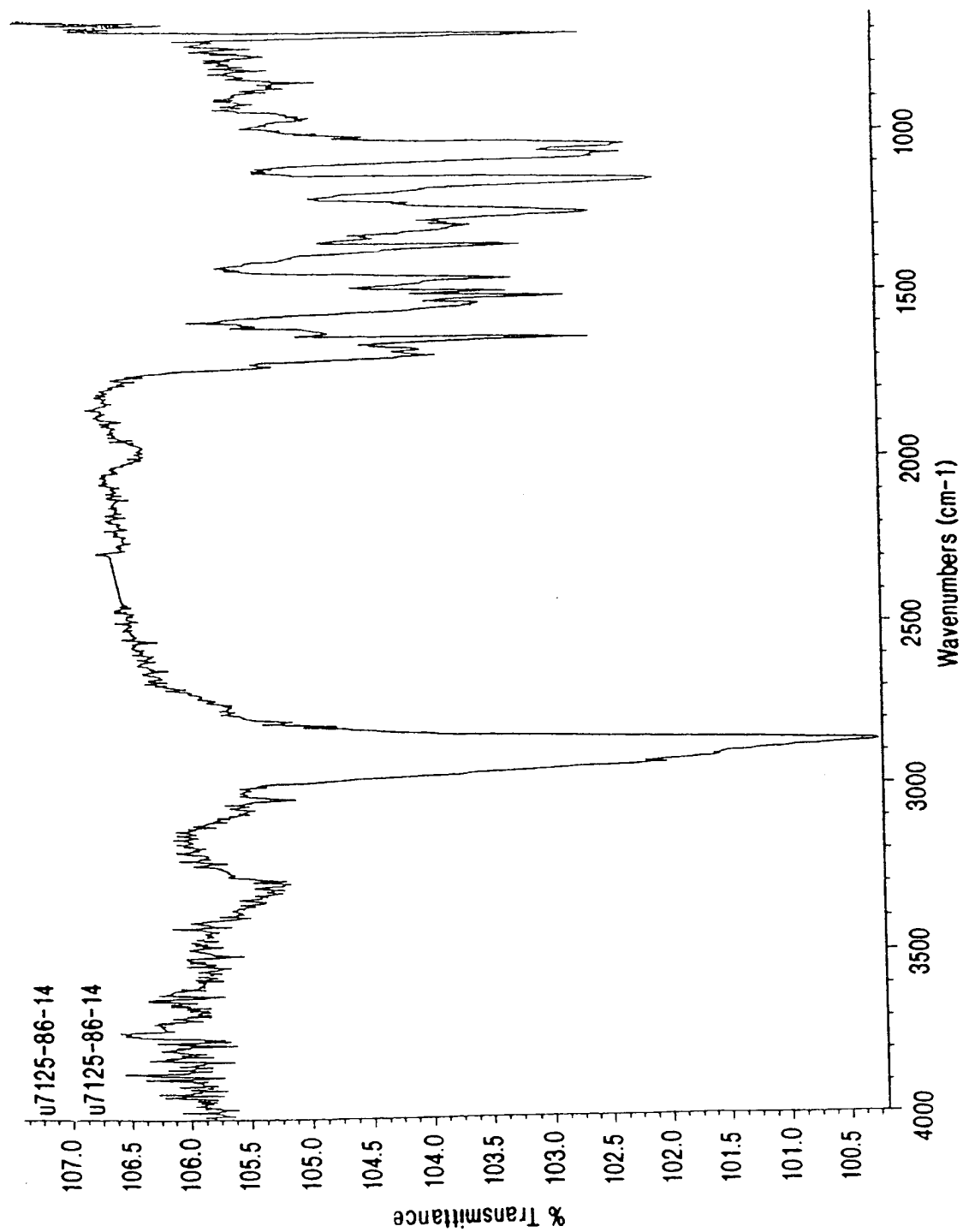


FIG. 31

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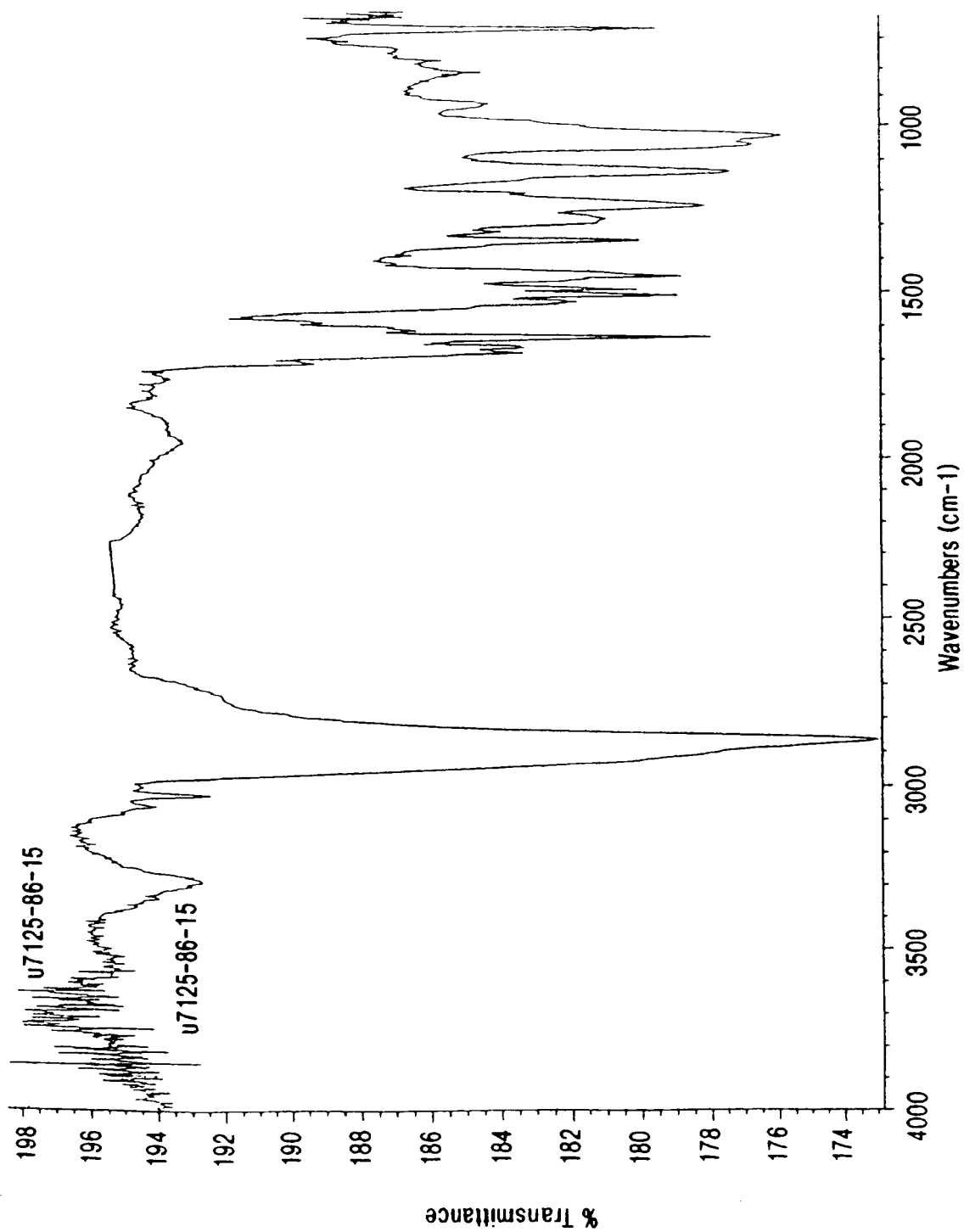


FIG. 32

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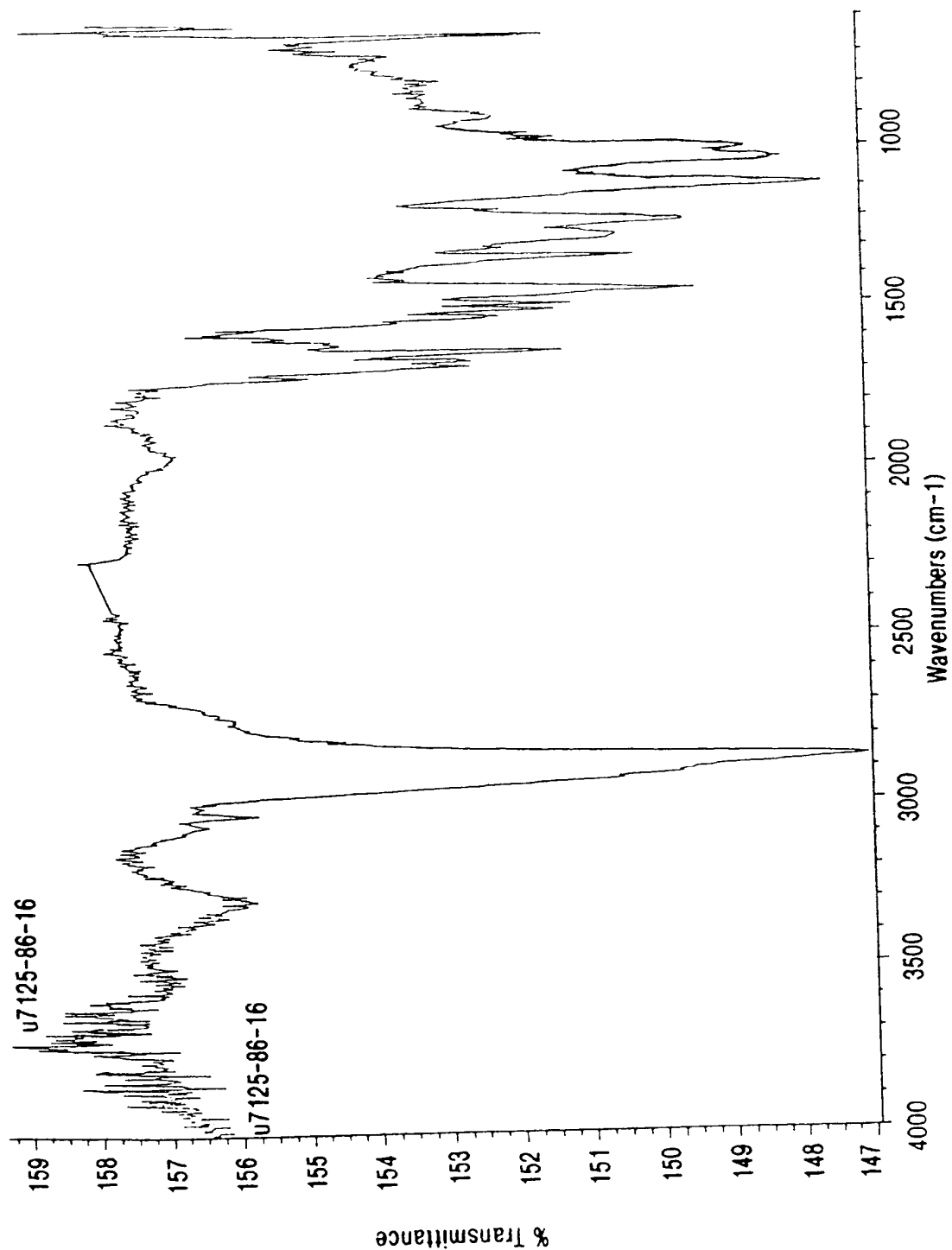


FIG. 33

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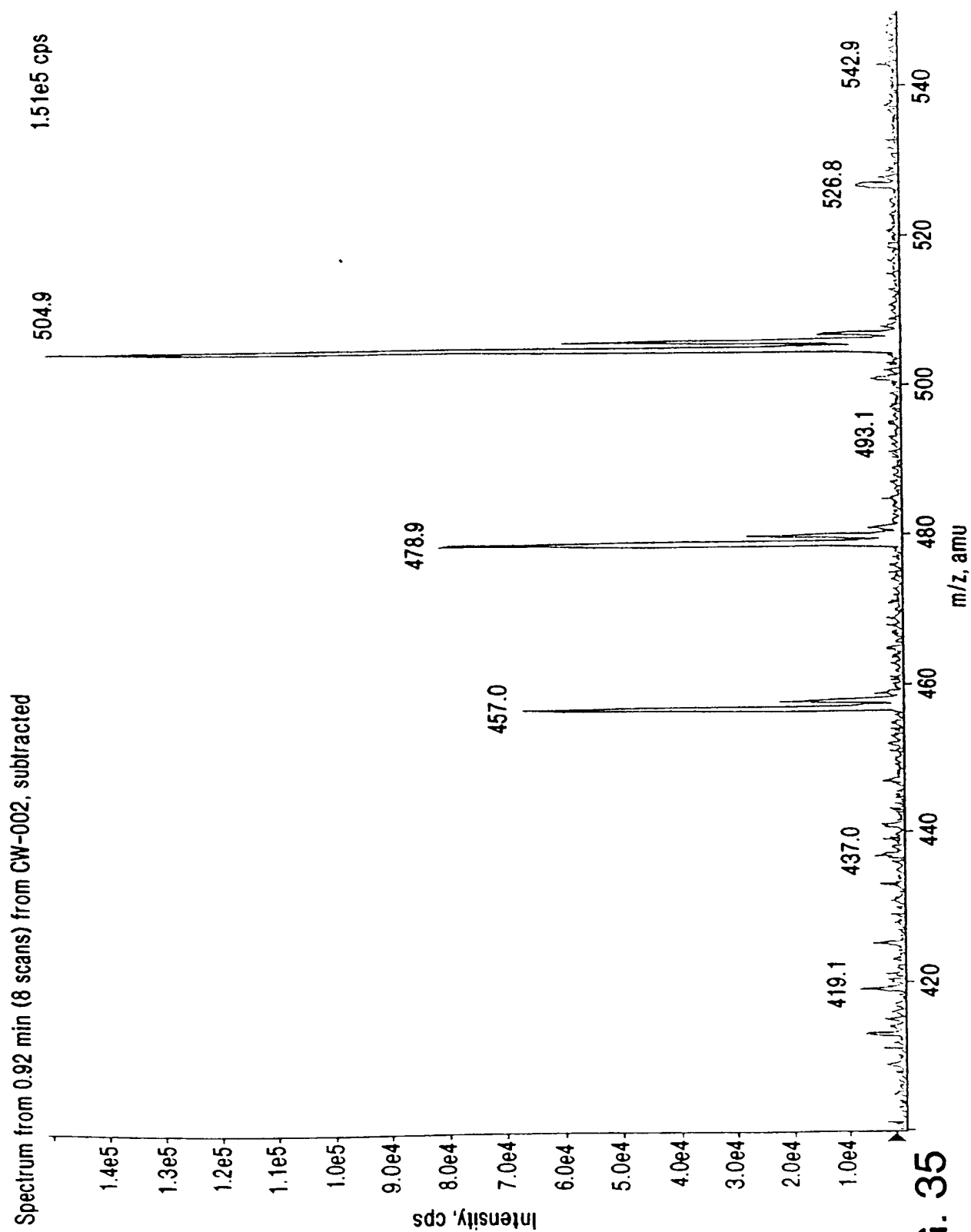


FIG. 35

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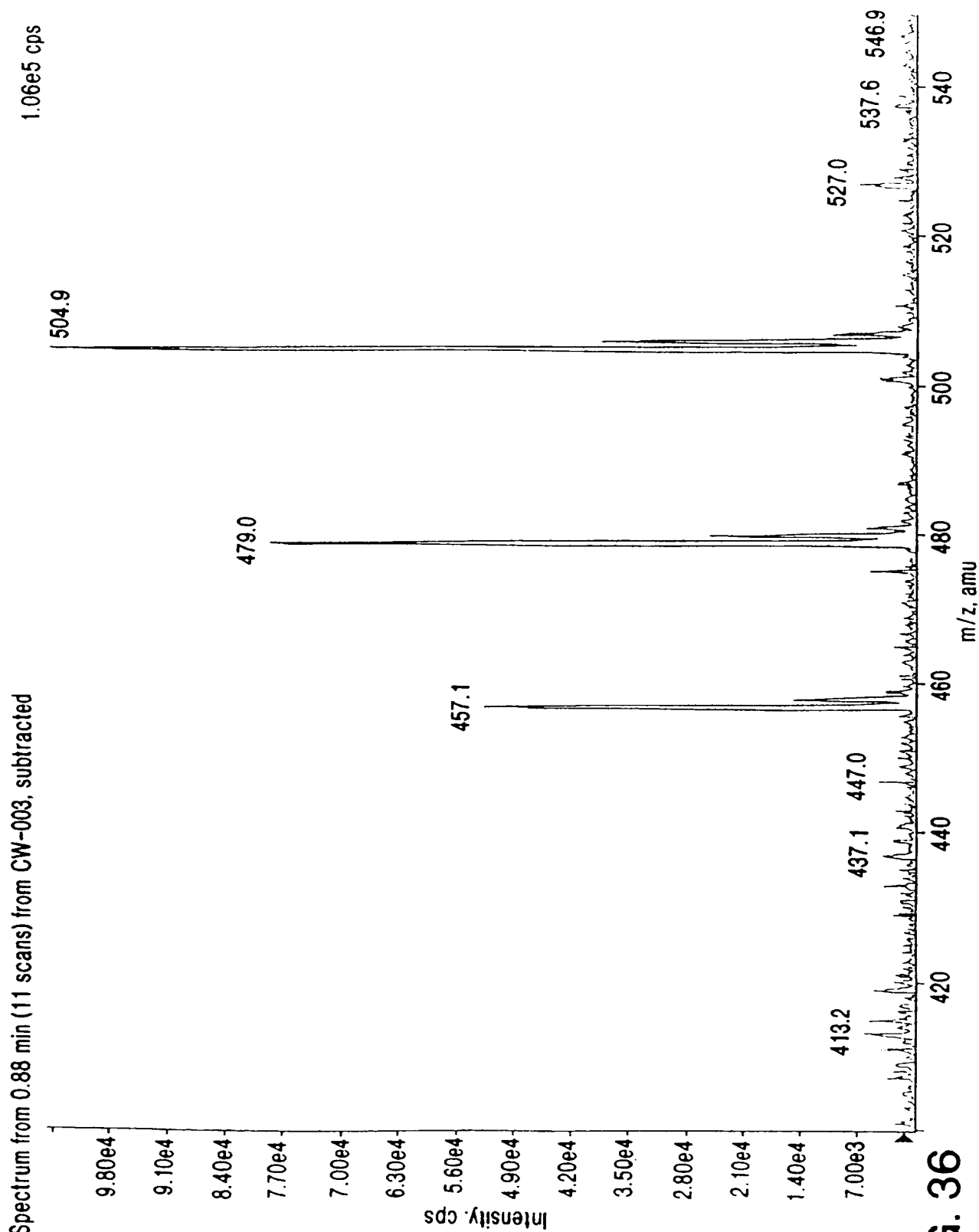


FIG. 36

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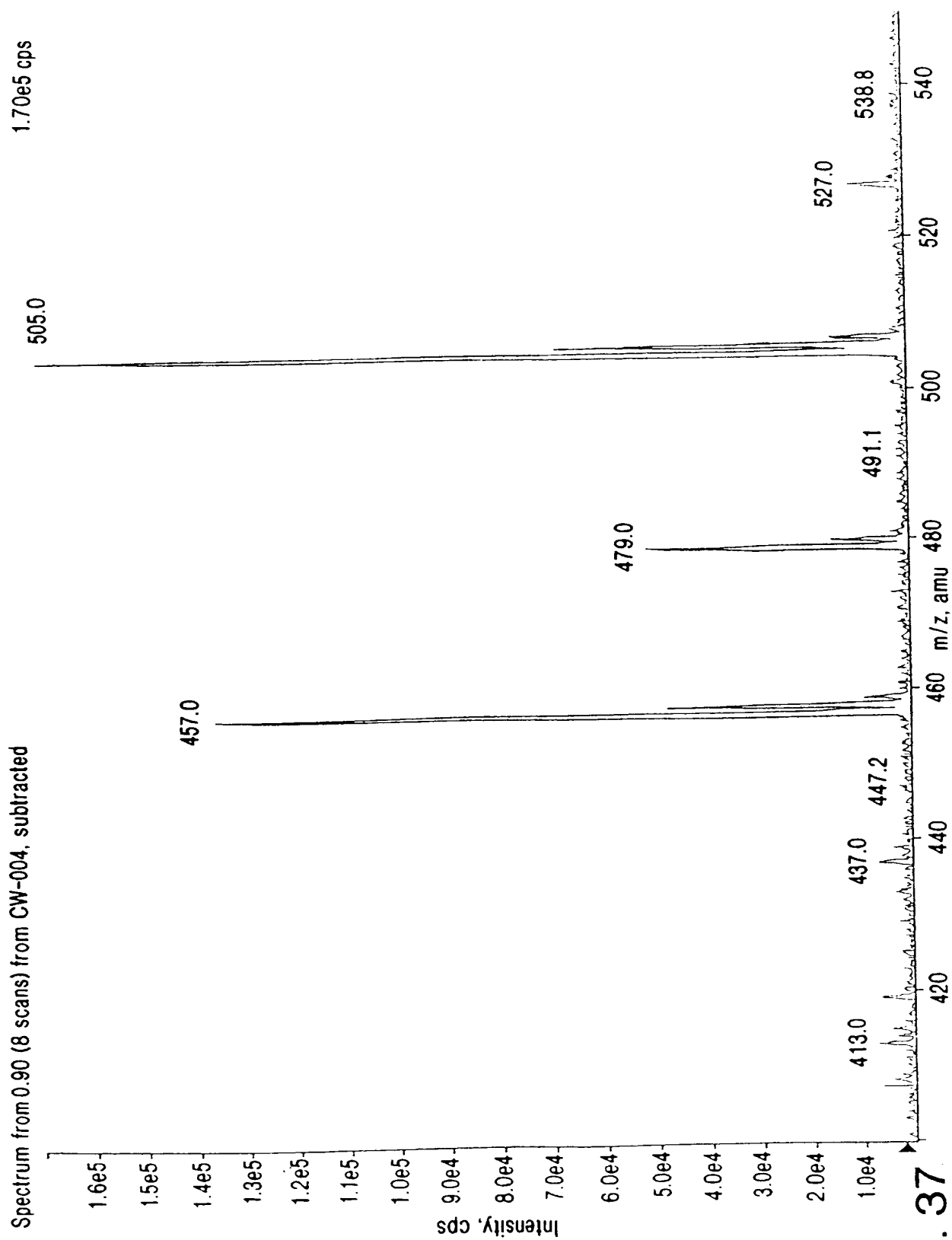


FIG. 37

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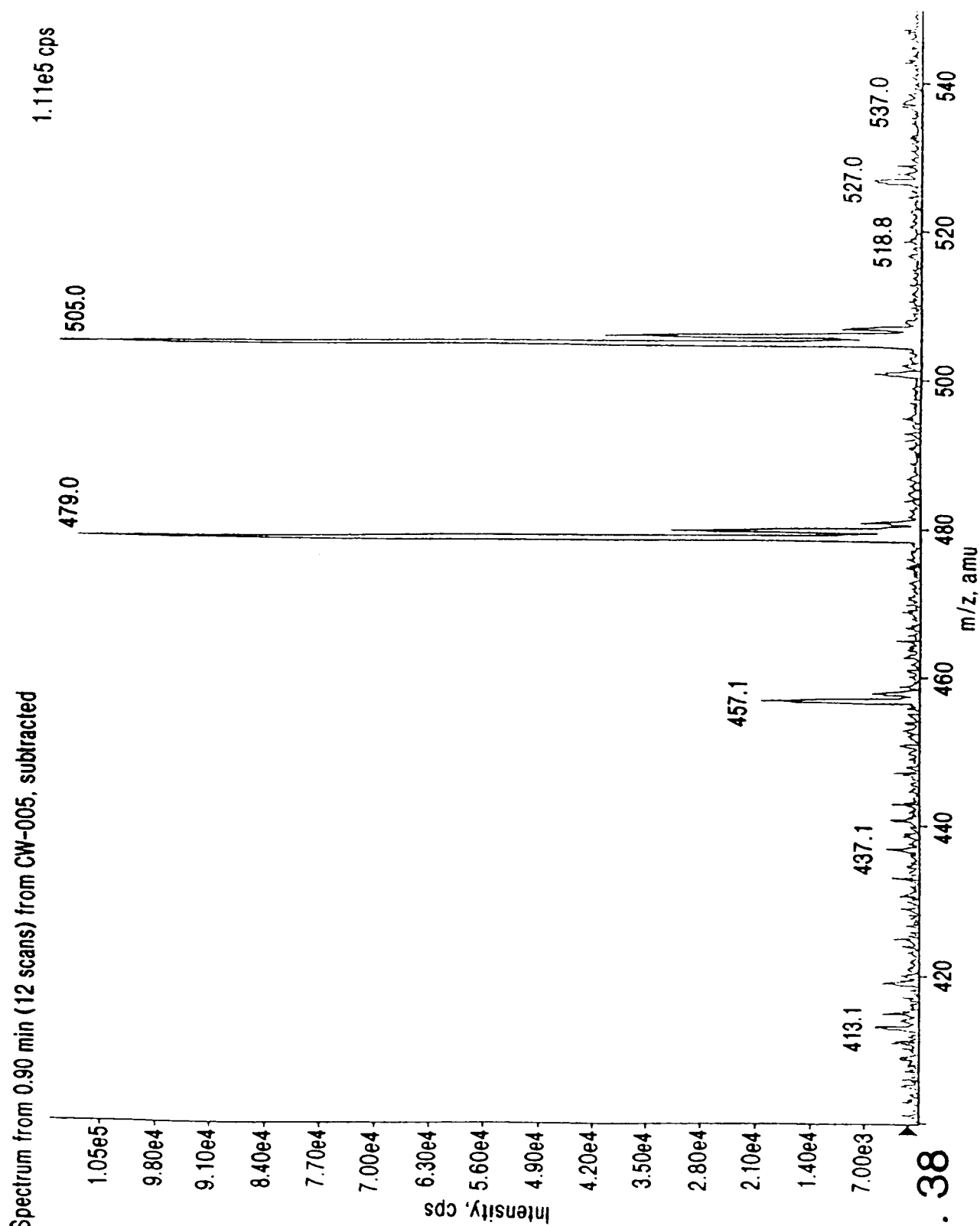


FIG. 38

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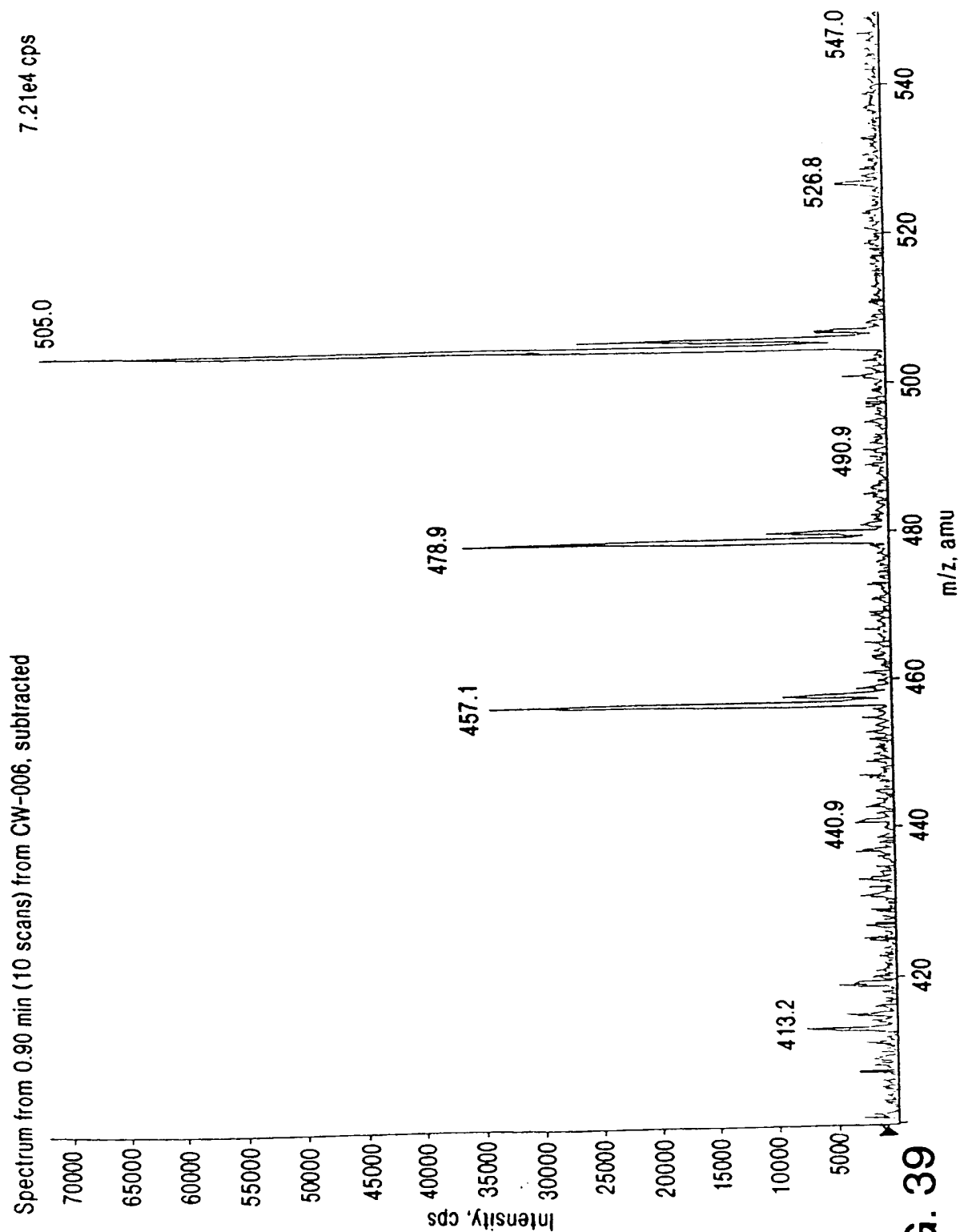


FIG. 39

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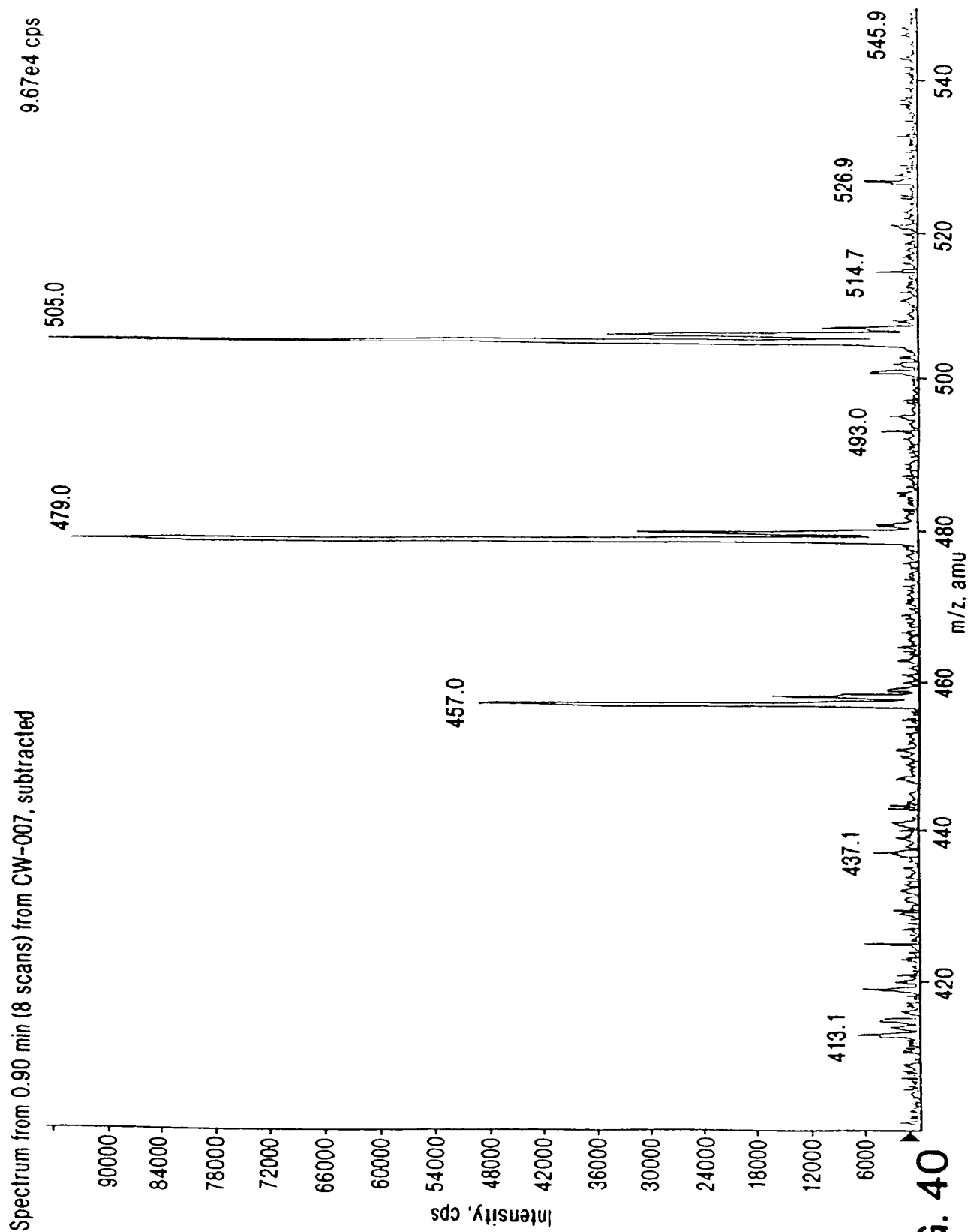


FIG. 40

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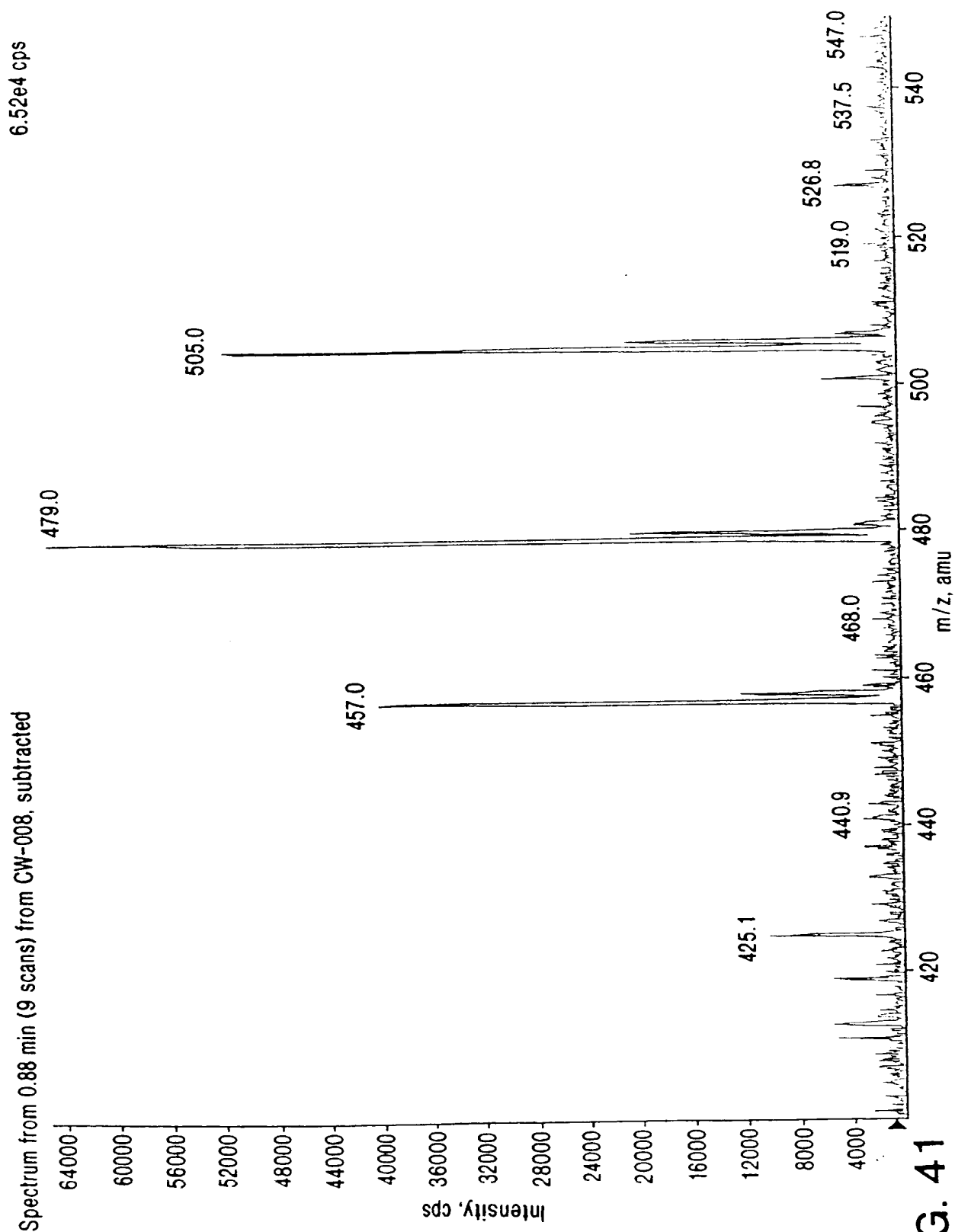


FIG. 41

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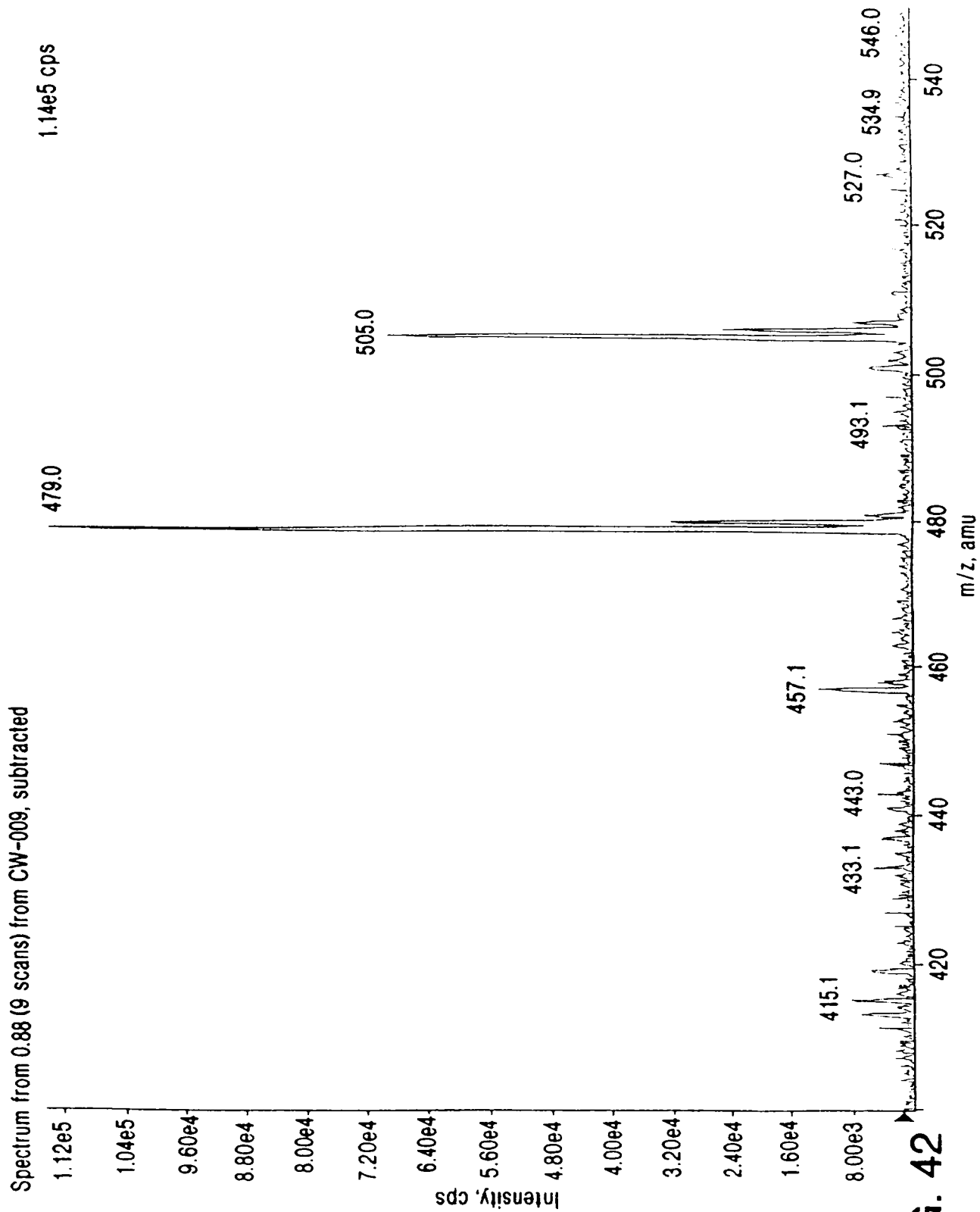


FIG. 42

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1.52e5 cps

Spectrum from 0.92 min (9 scans) from CW-010, subtracted

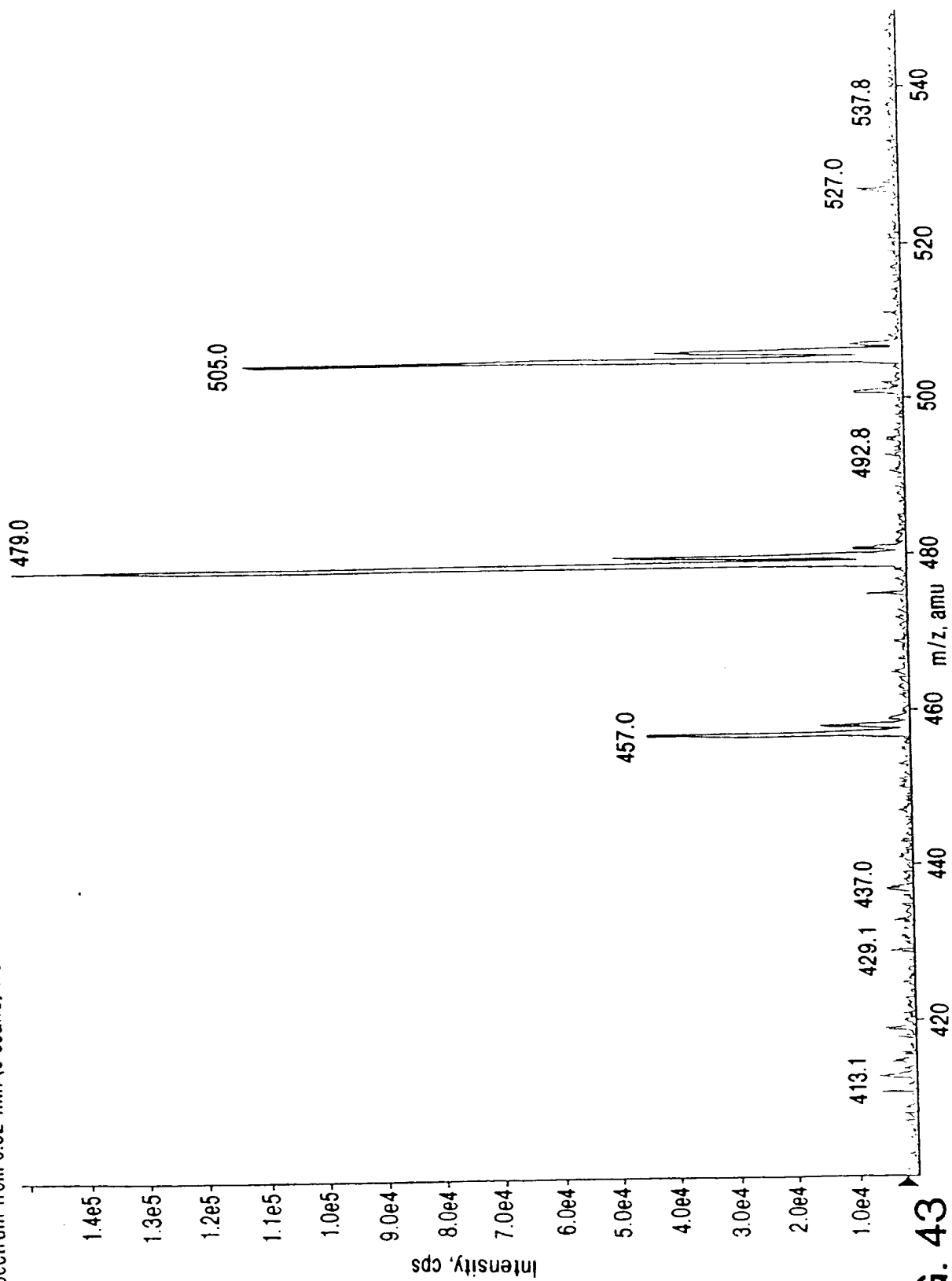


FIG. 43

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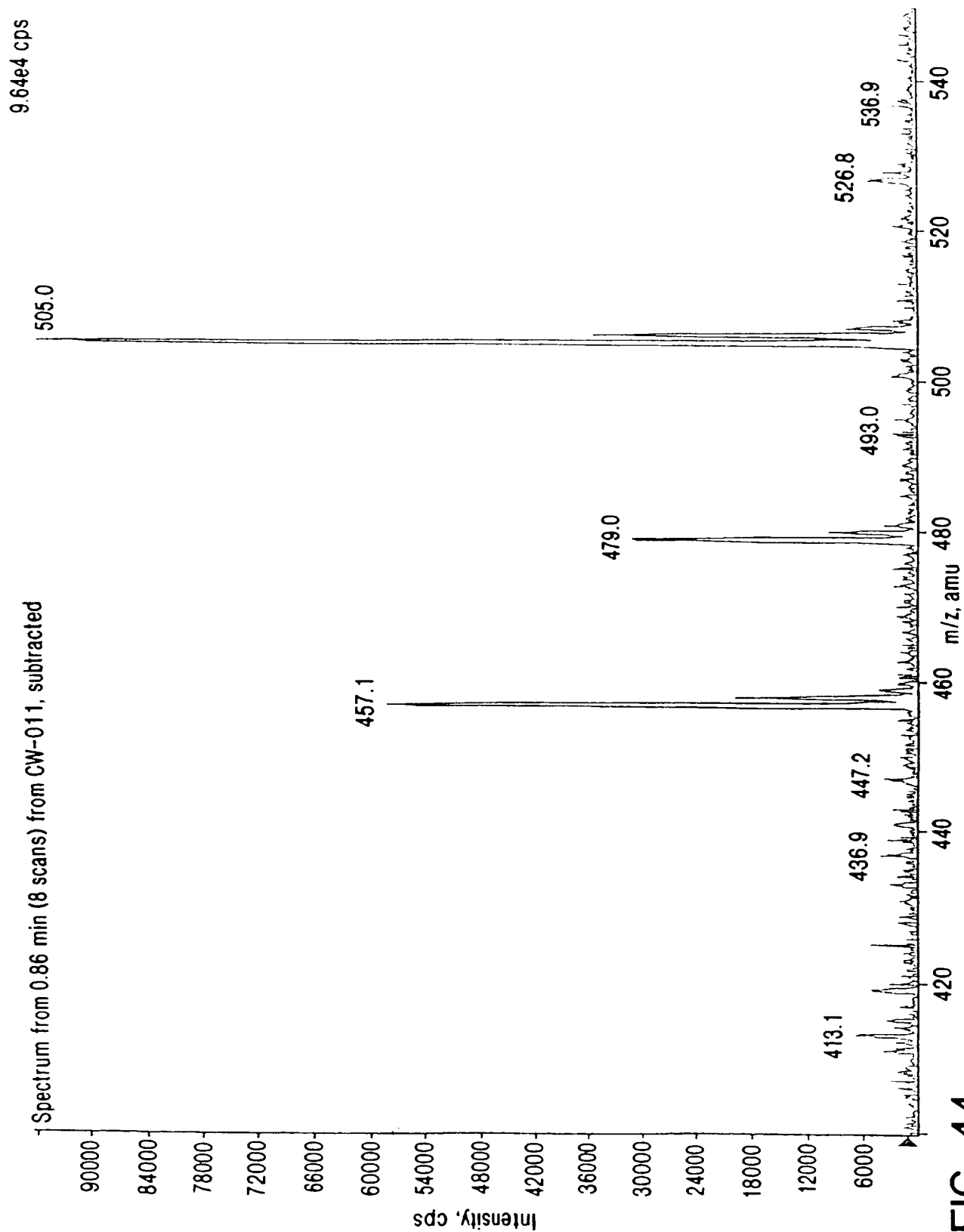


FIG. 44

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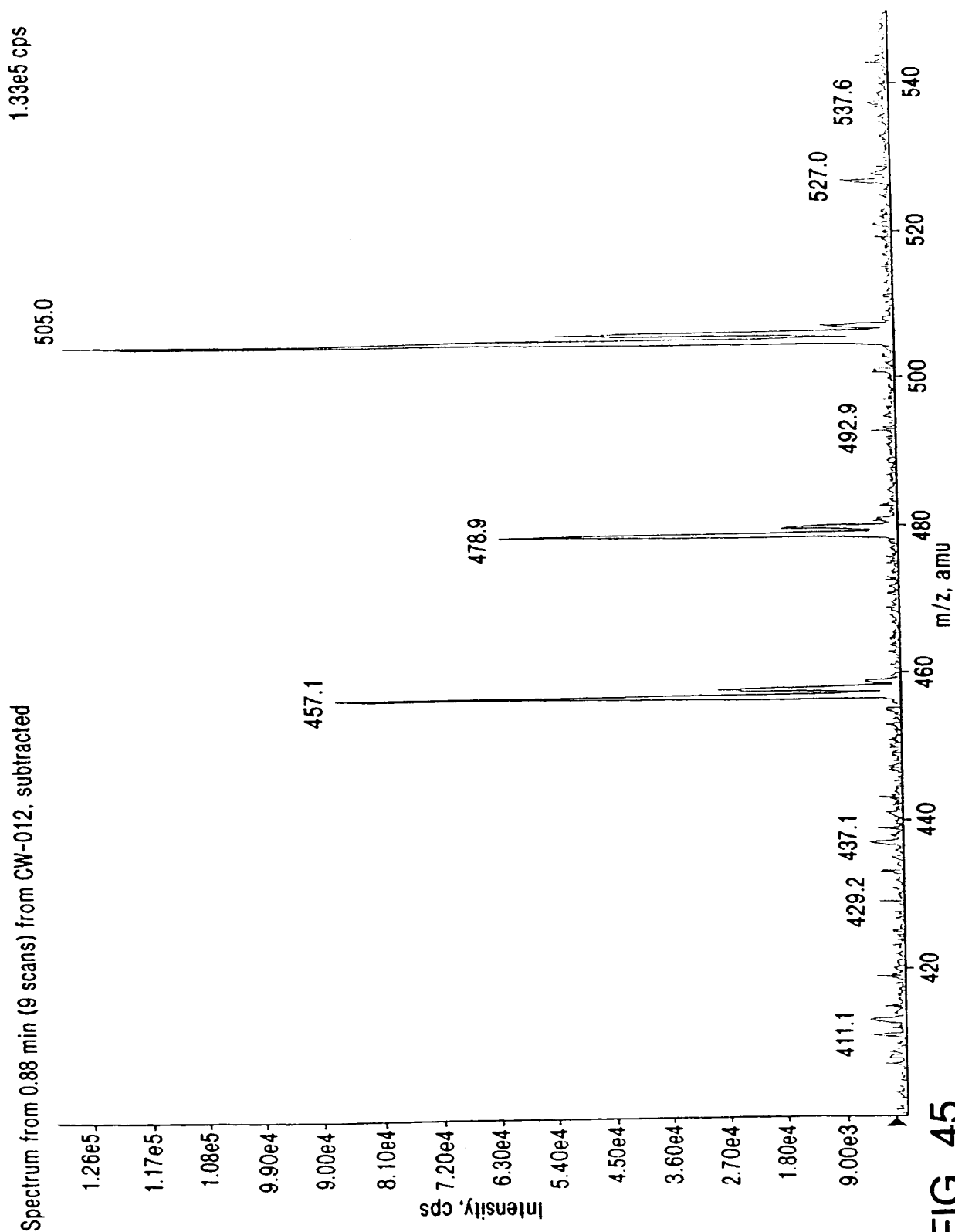


FIG. 45

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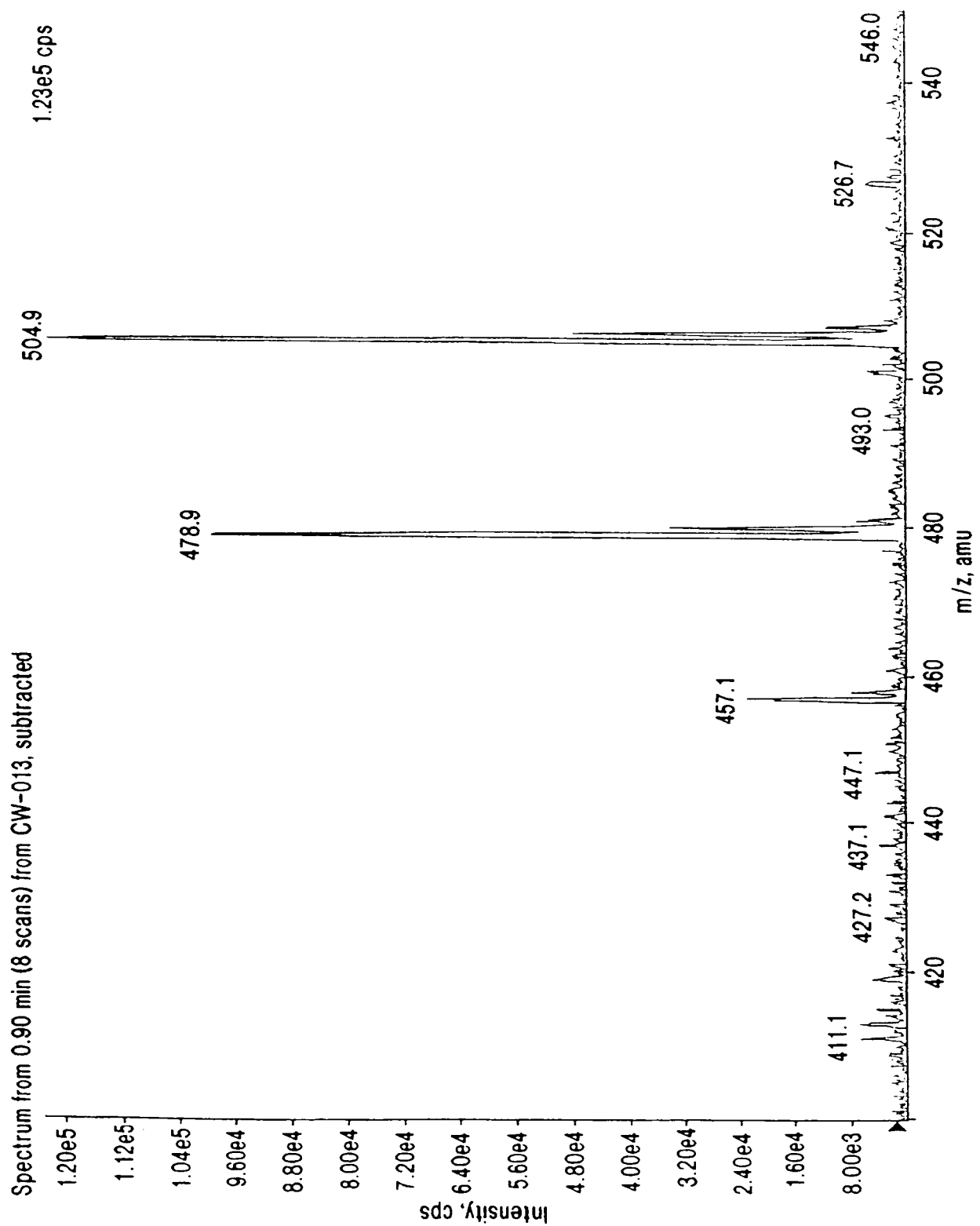


FIG. 46

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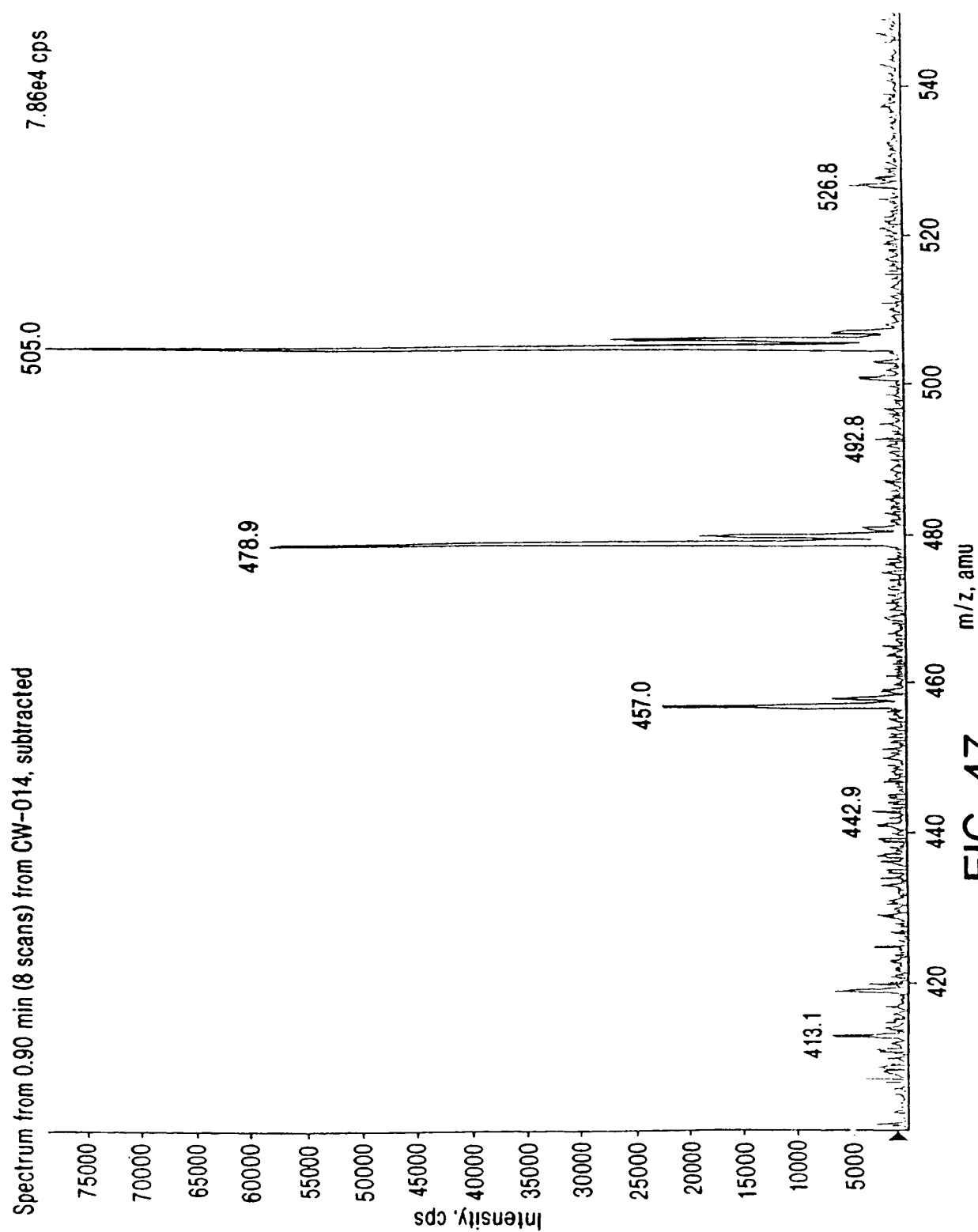


FIG. 47

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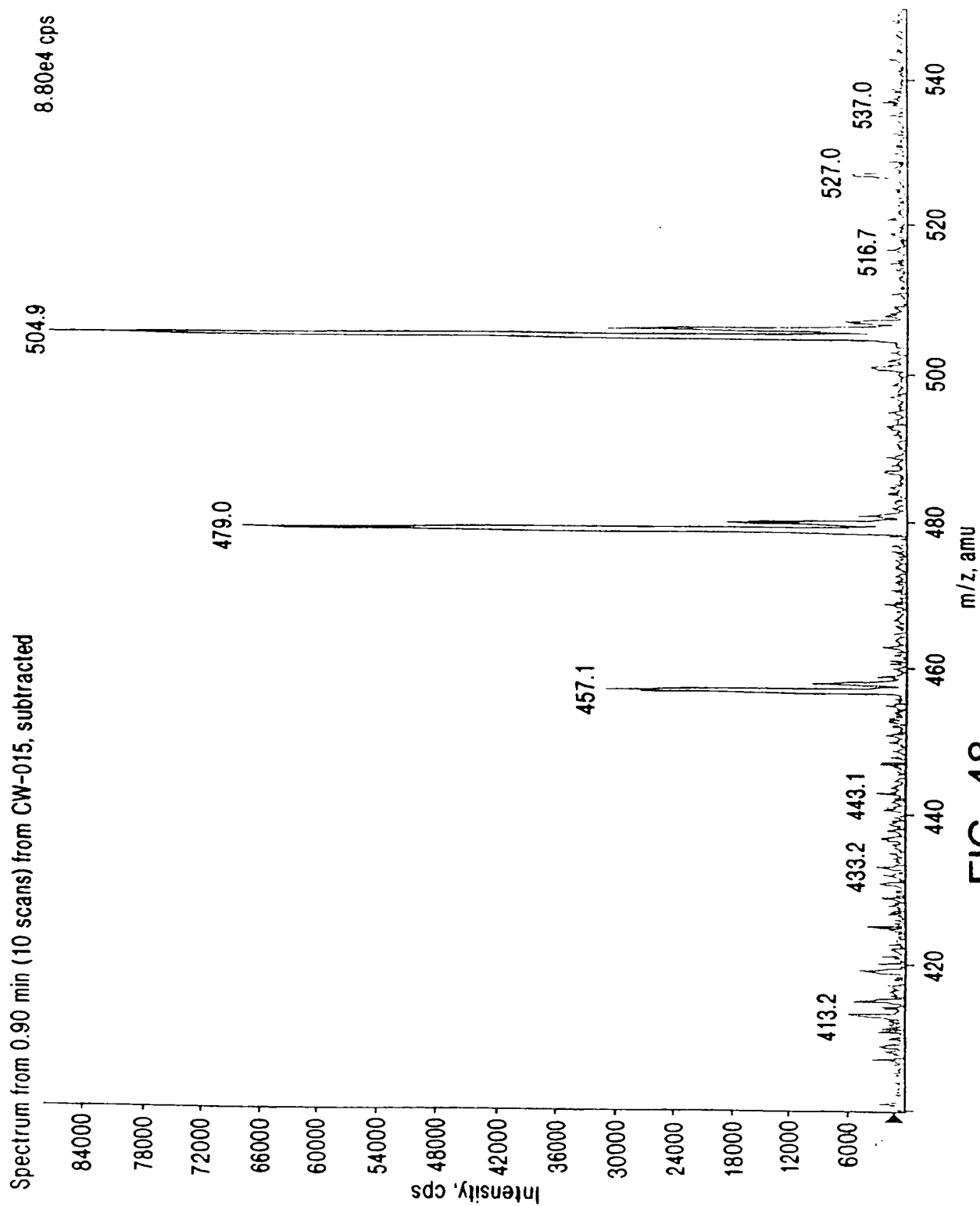


FIG. 48

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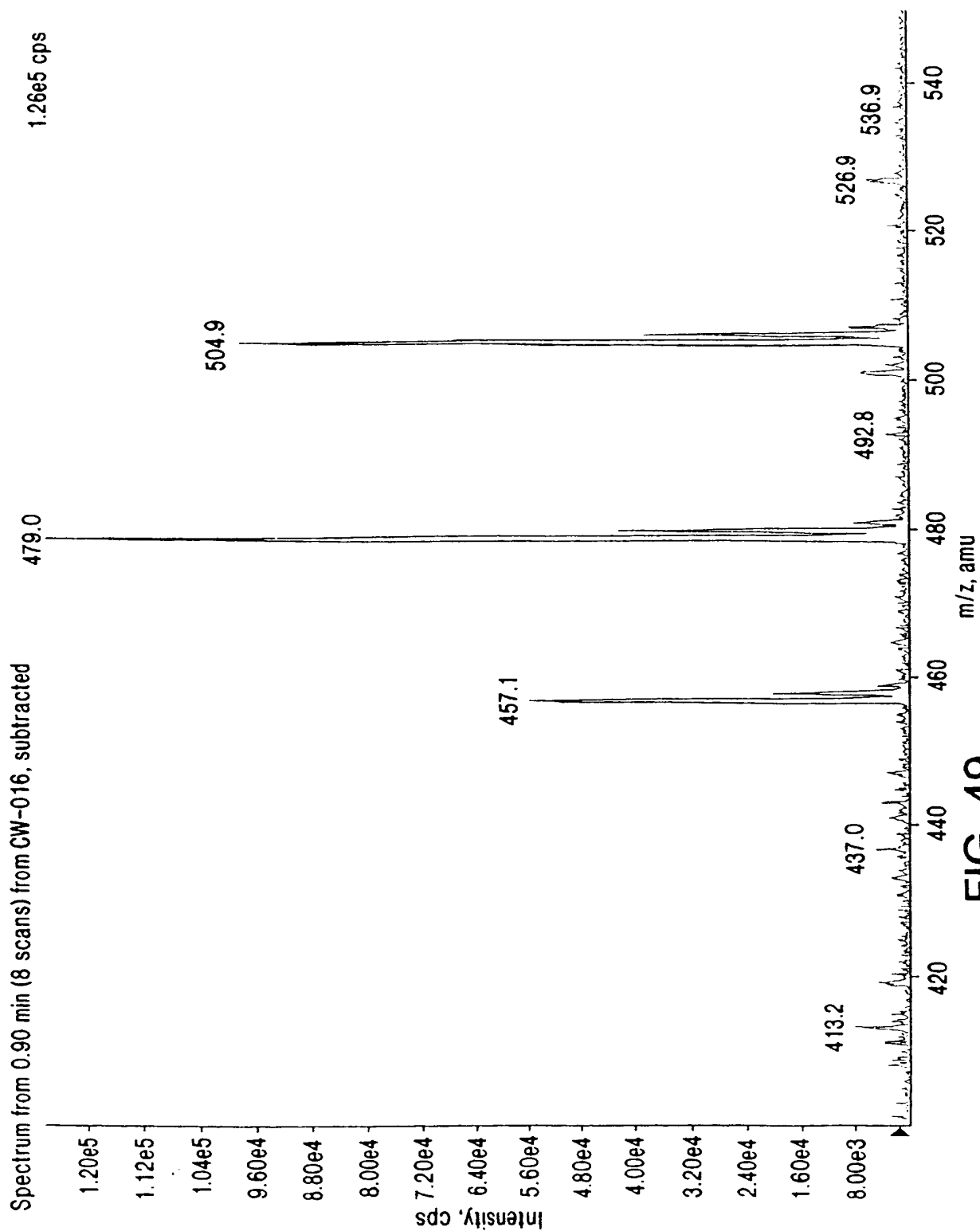


FIG. 49

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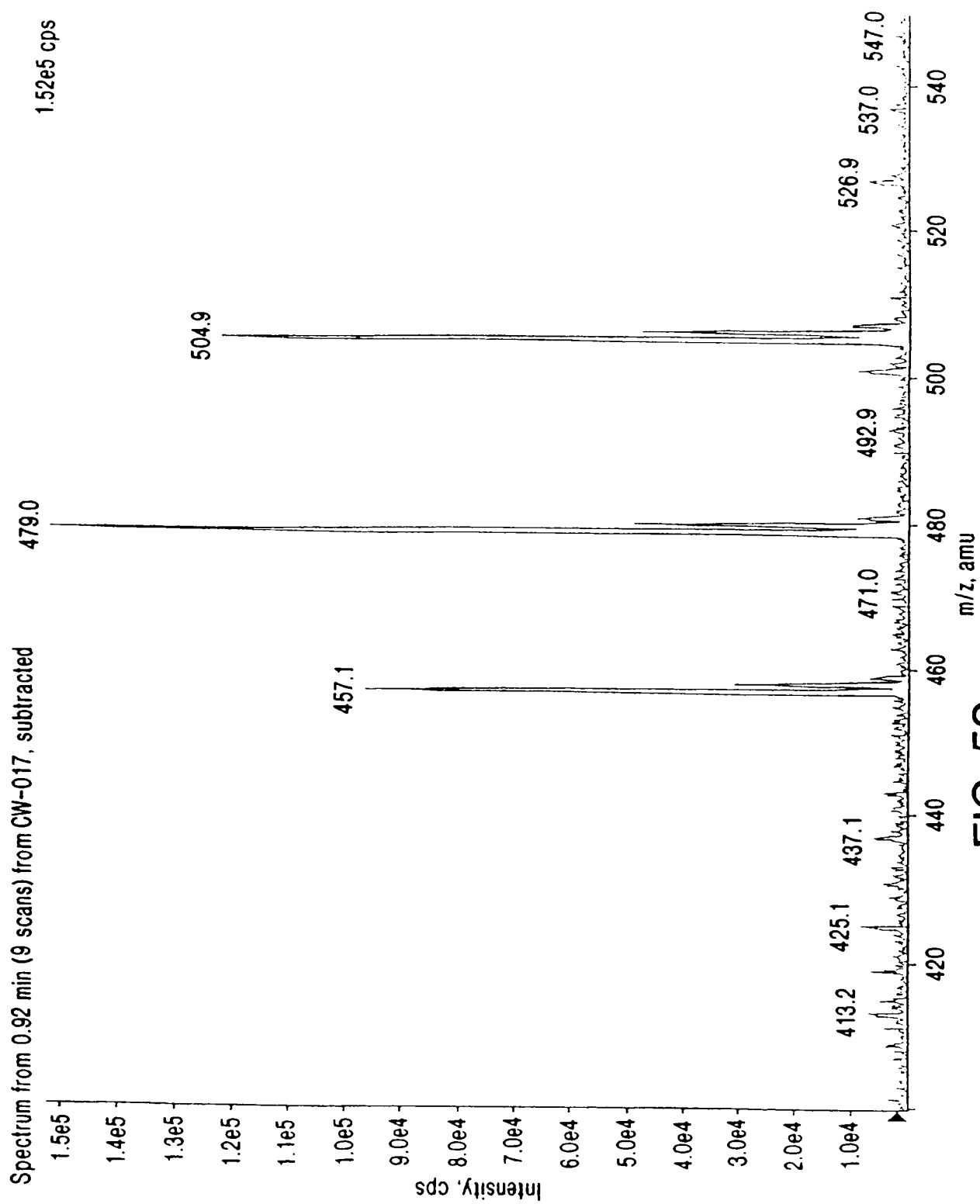


FIG. 50

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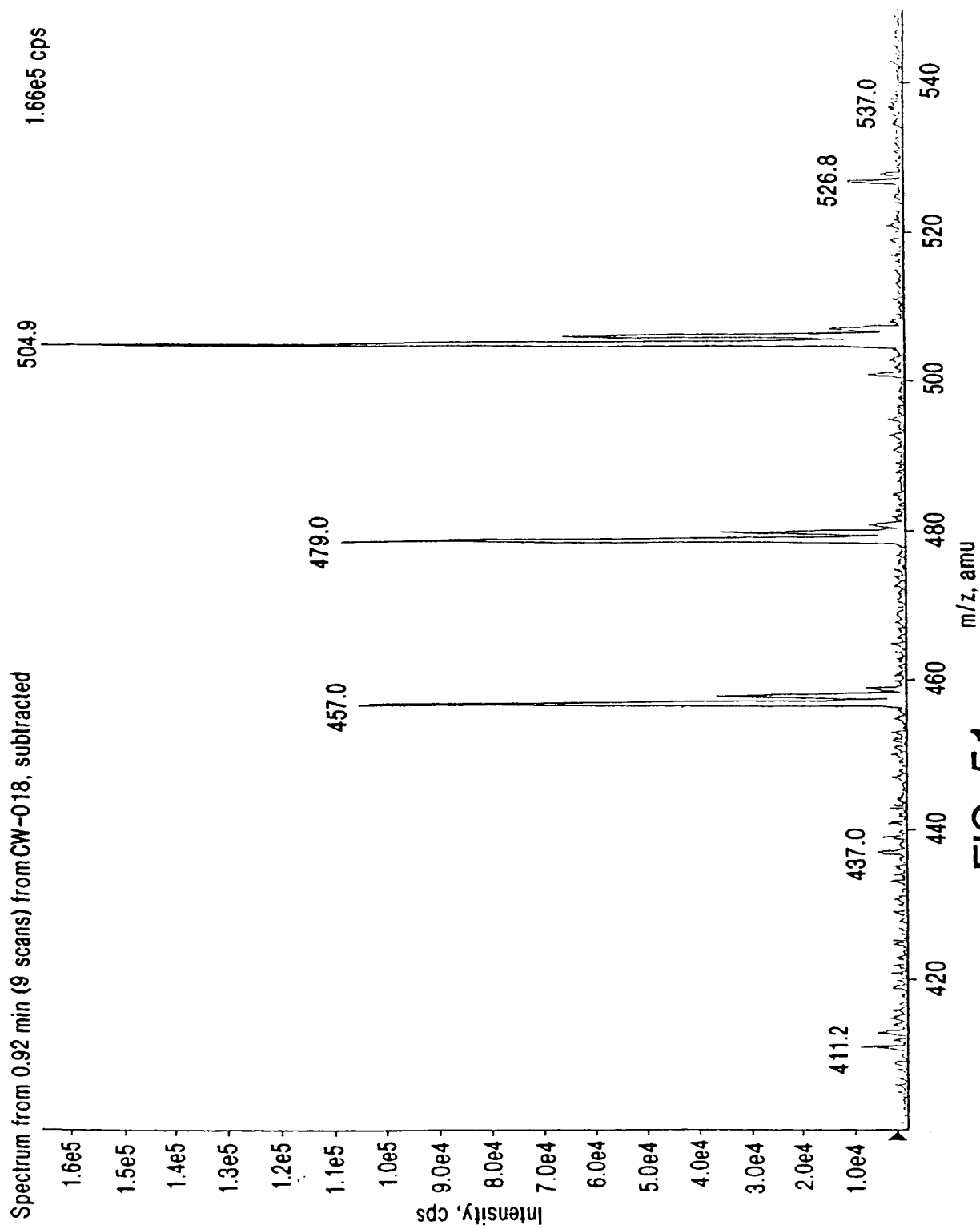


FIG. 51

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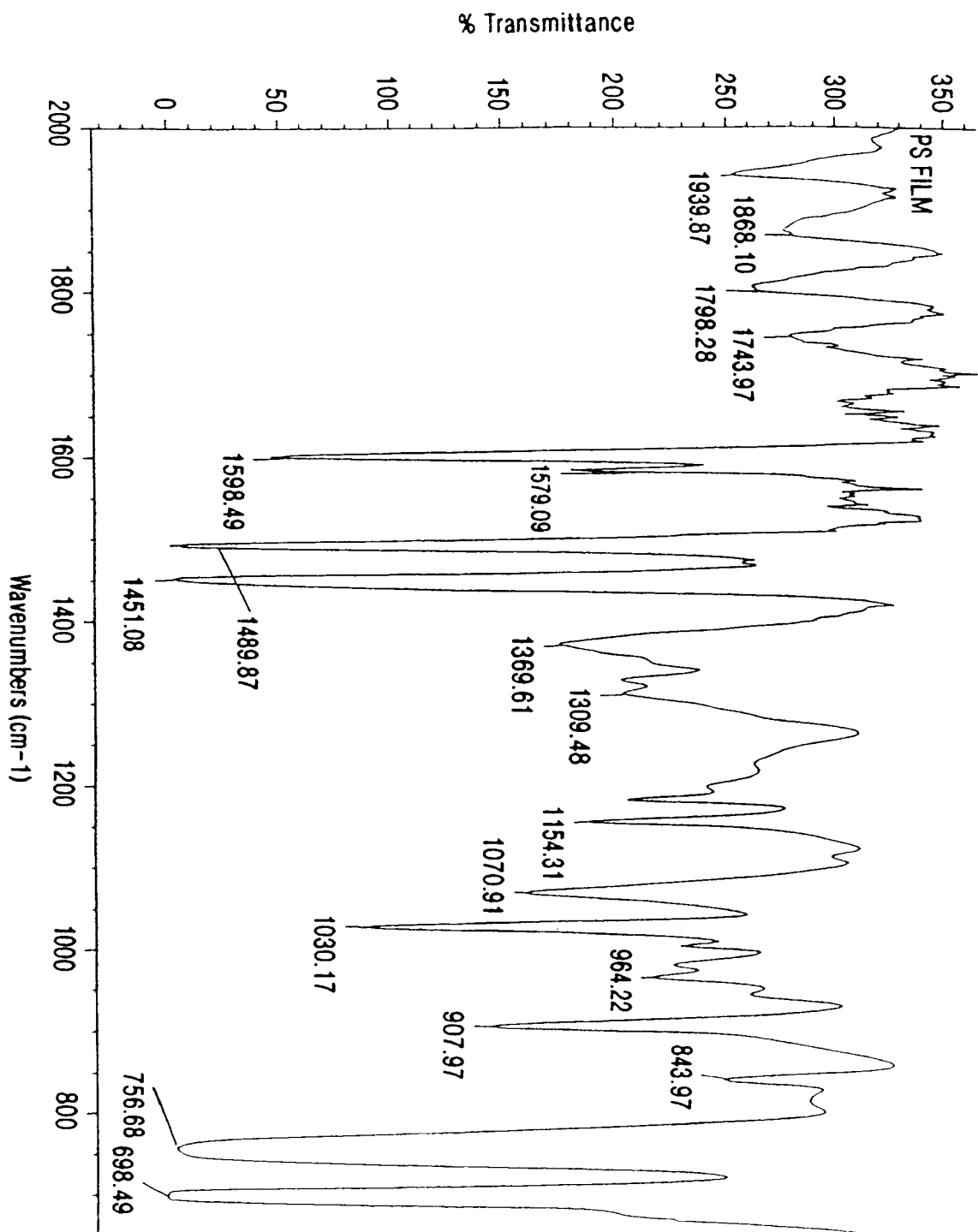


FIG. 52

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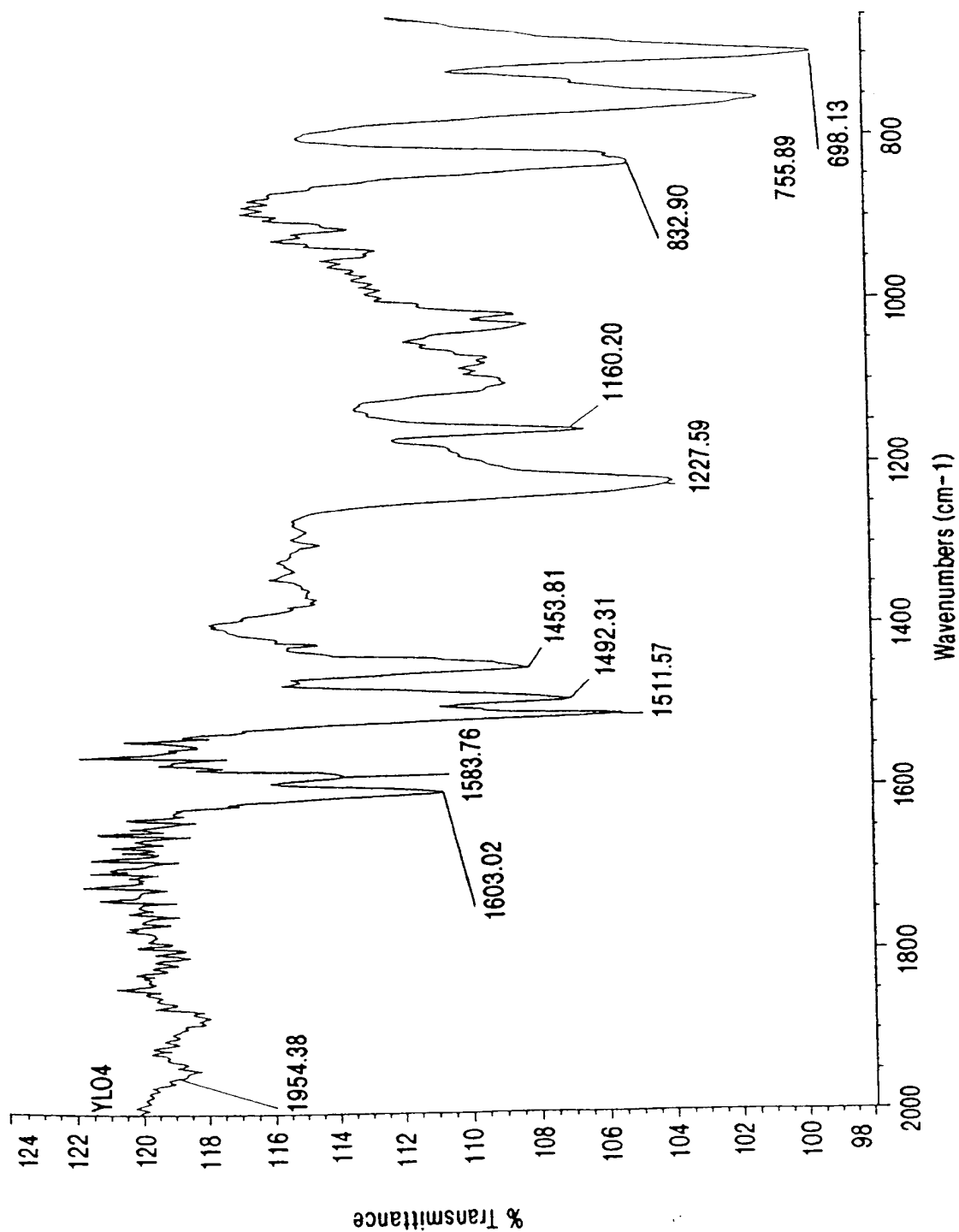


FIG. 53

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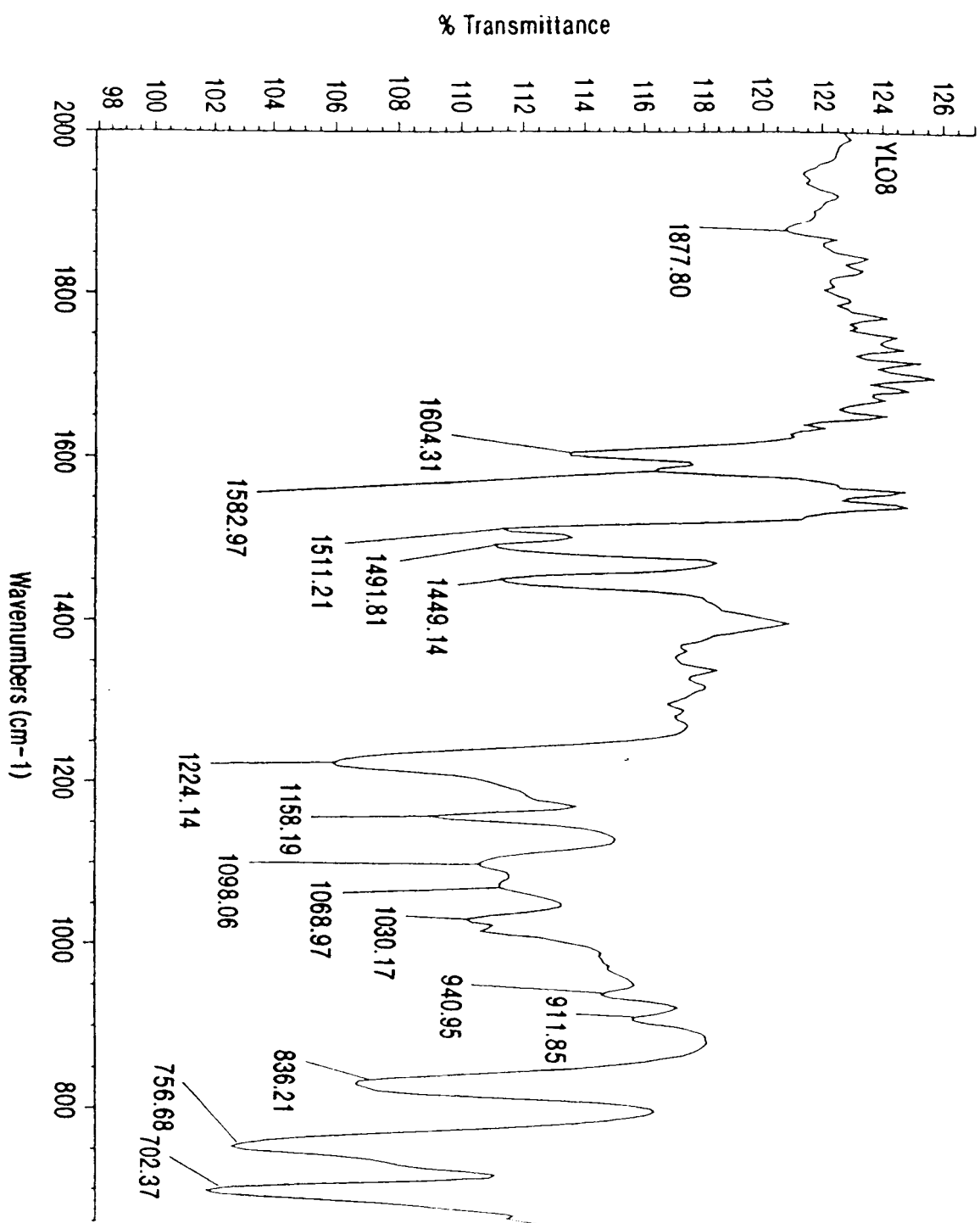


FIG. 54

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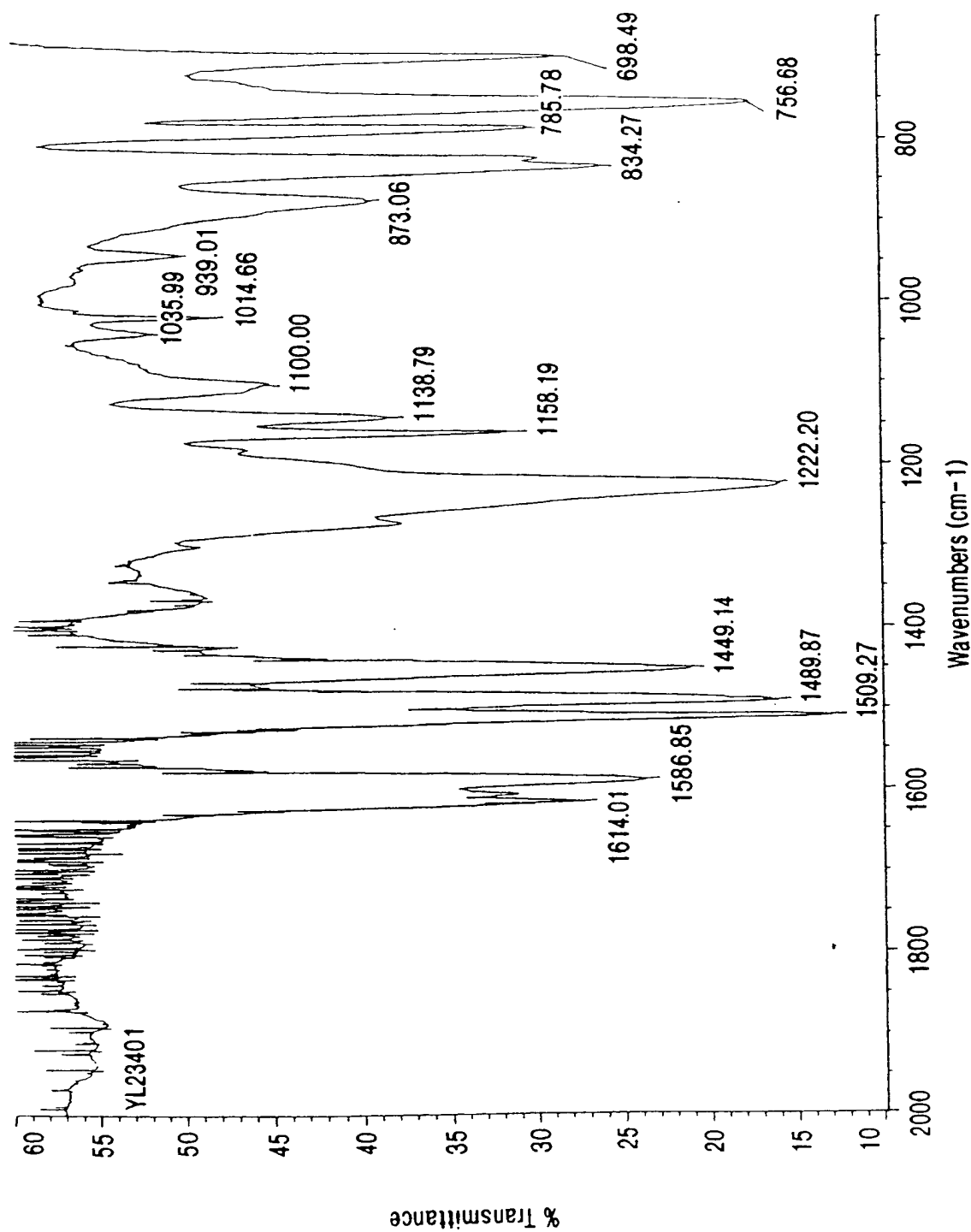


FIG. 55

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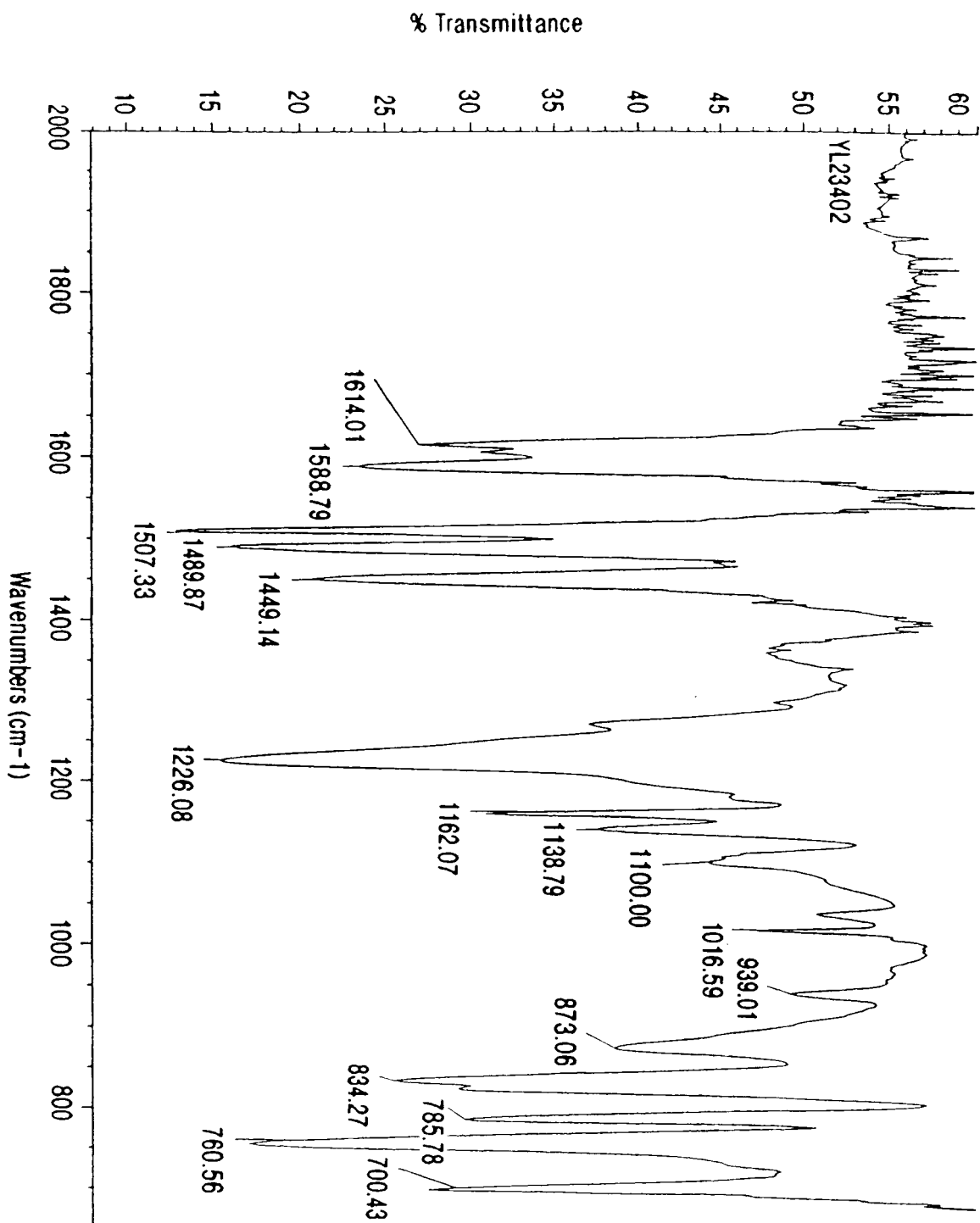


FIG. 56

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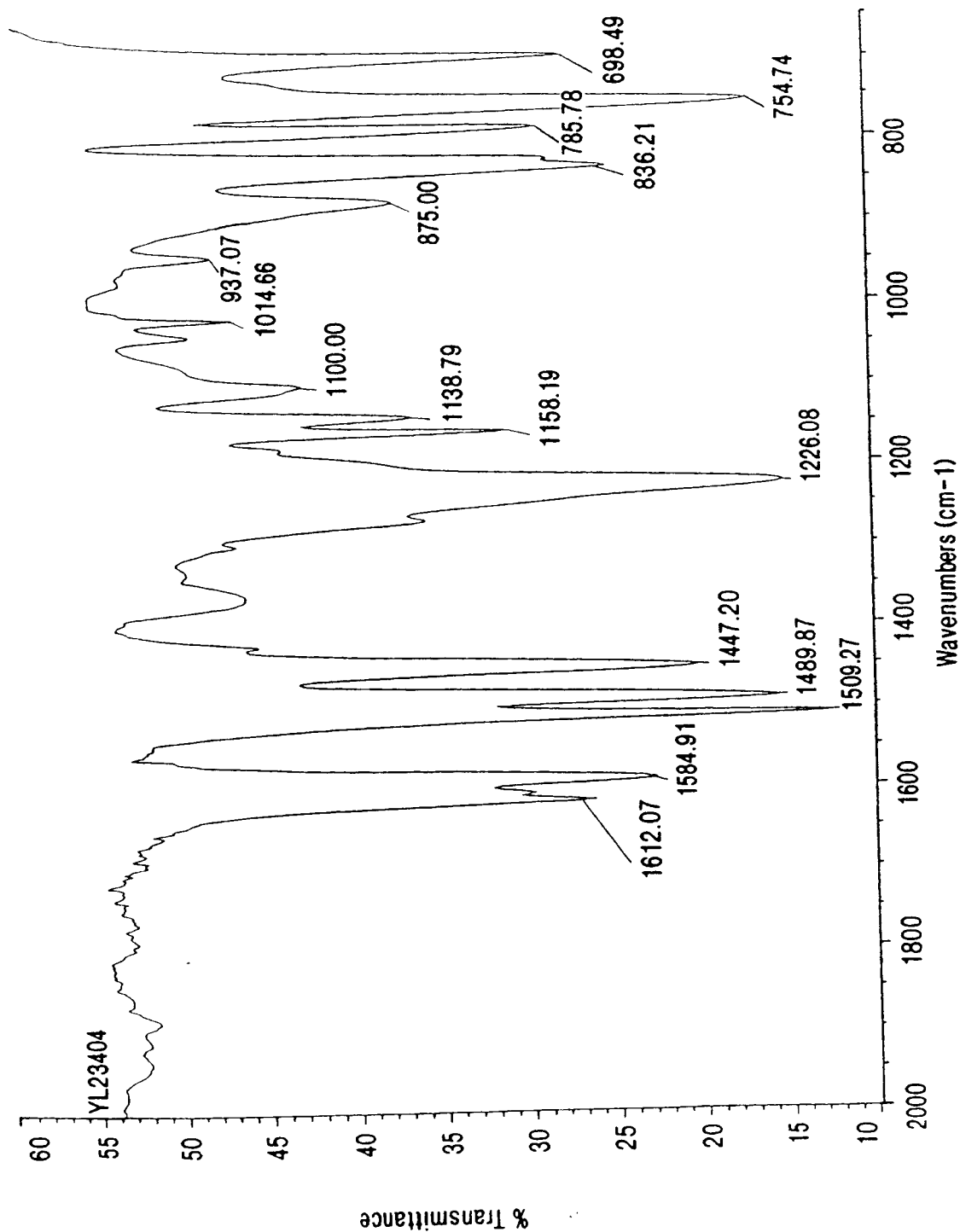


FIG. 57

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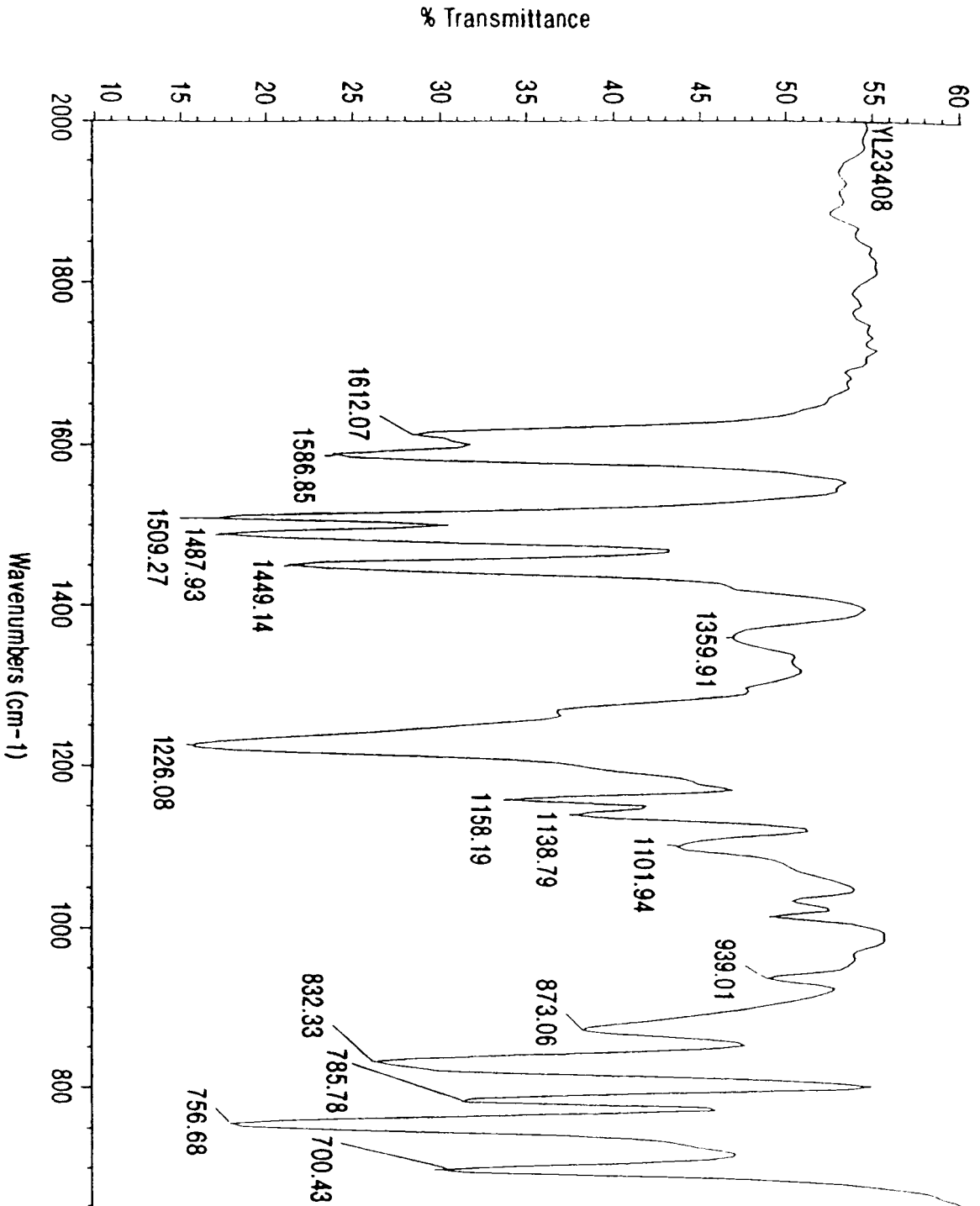


FIG. 58

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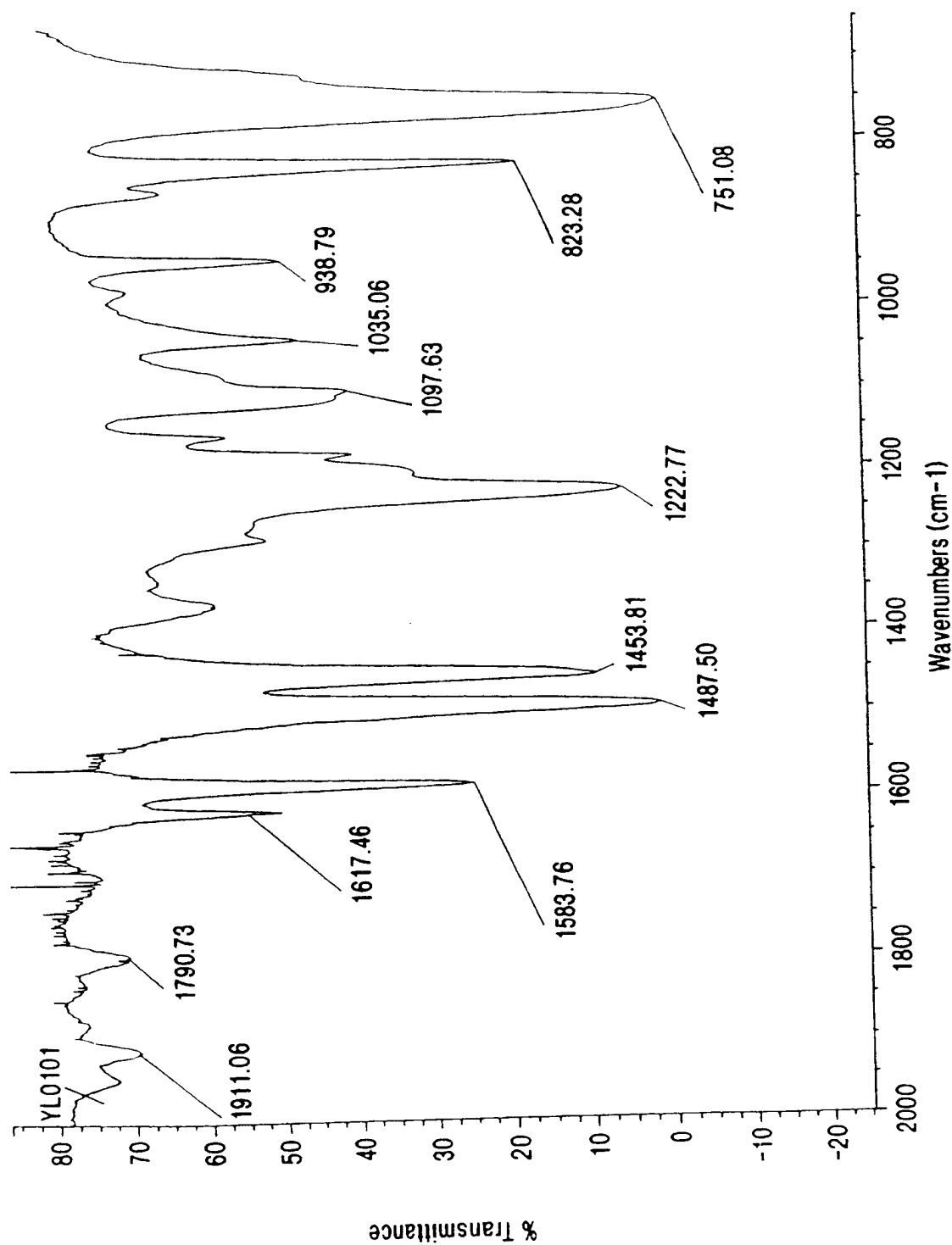
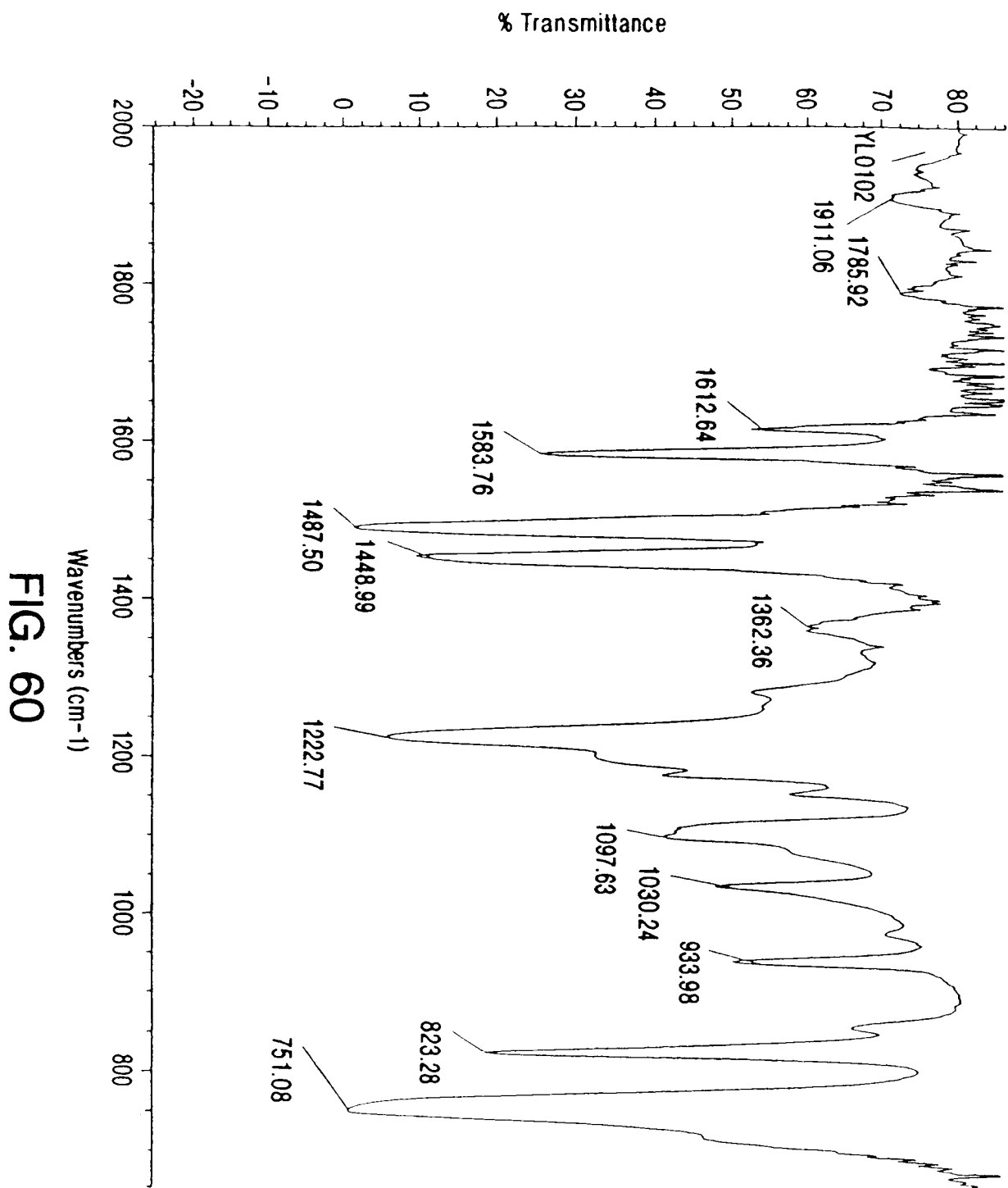


FIG. 59

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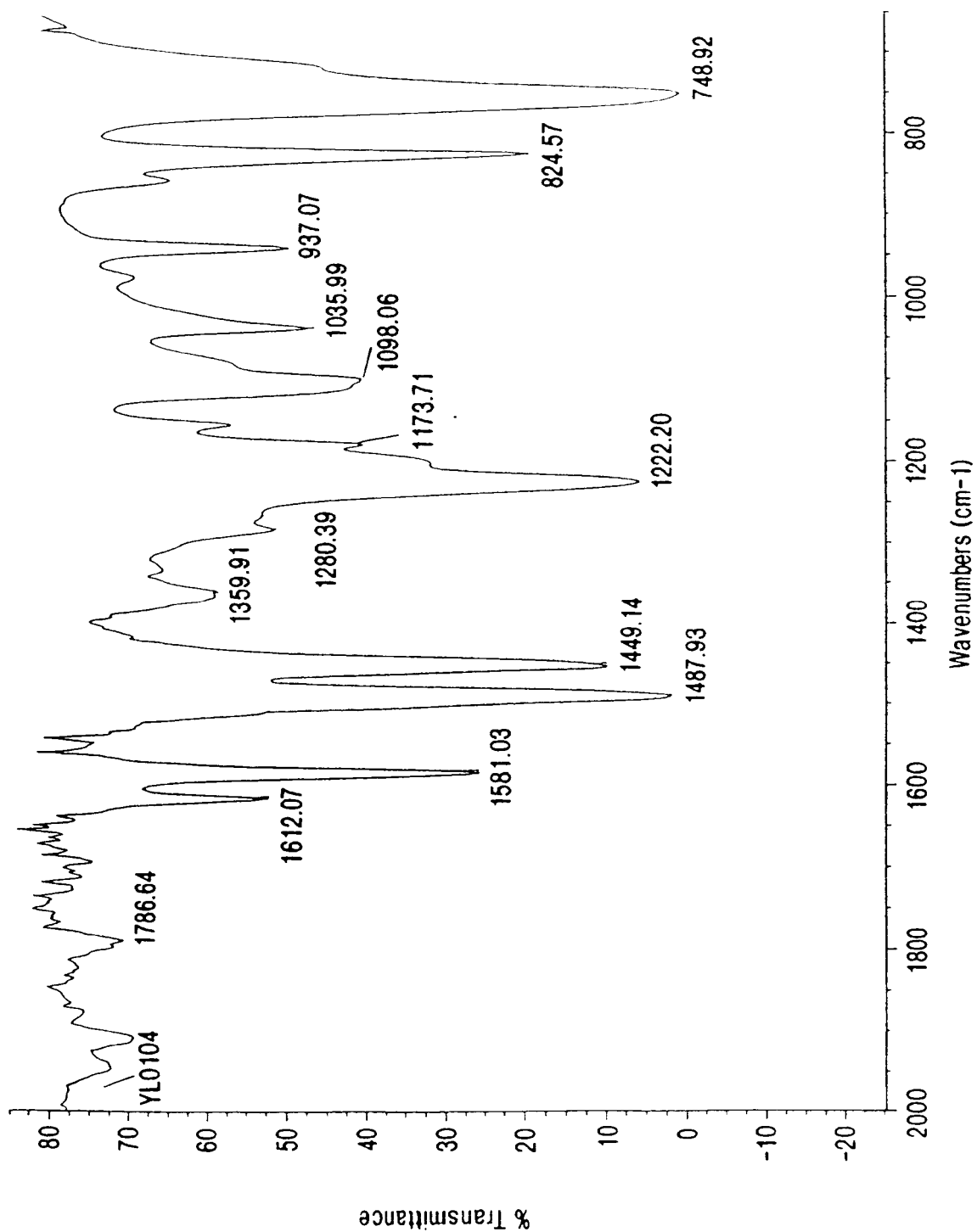


FIG. 61

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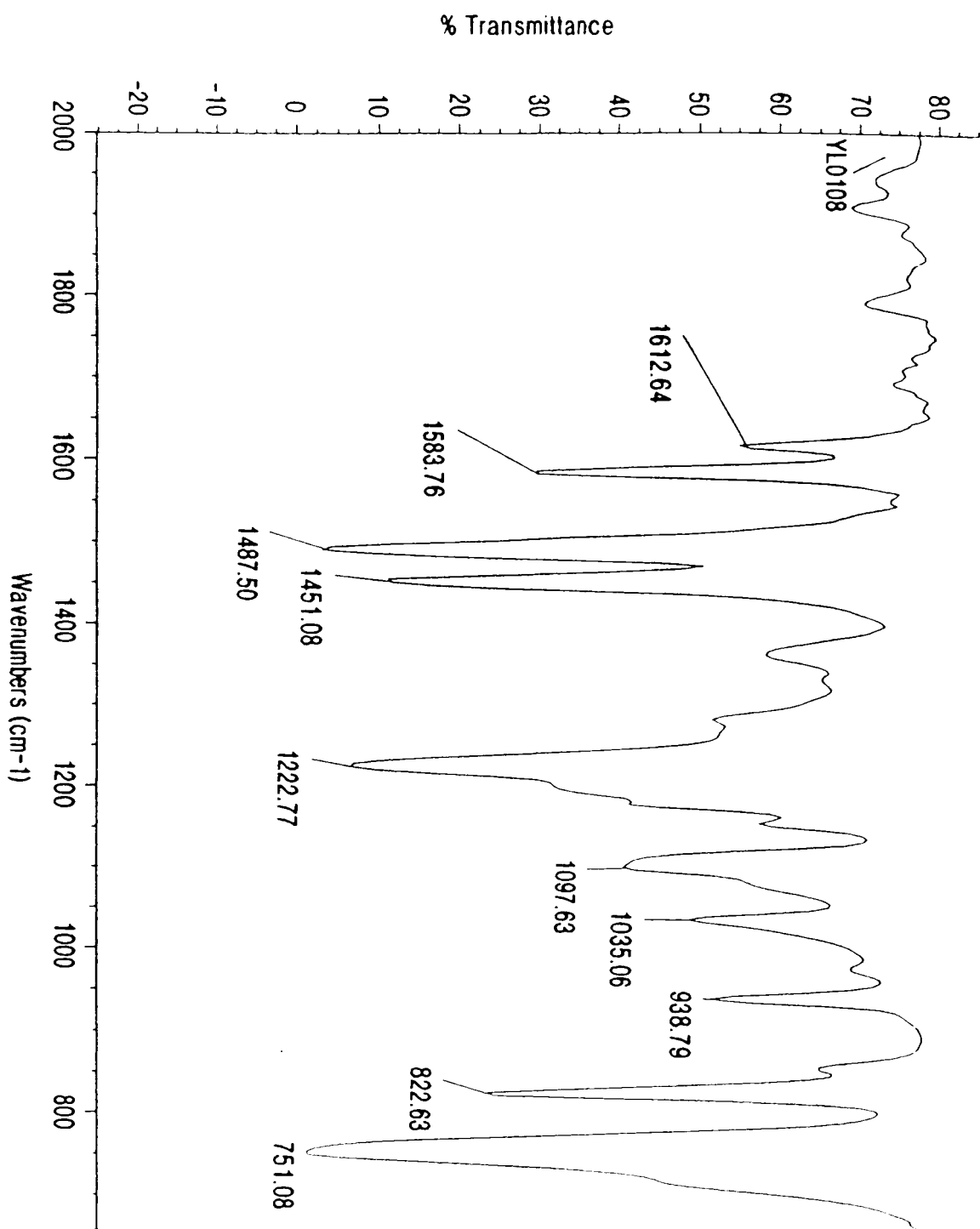


FIG. 62

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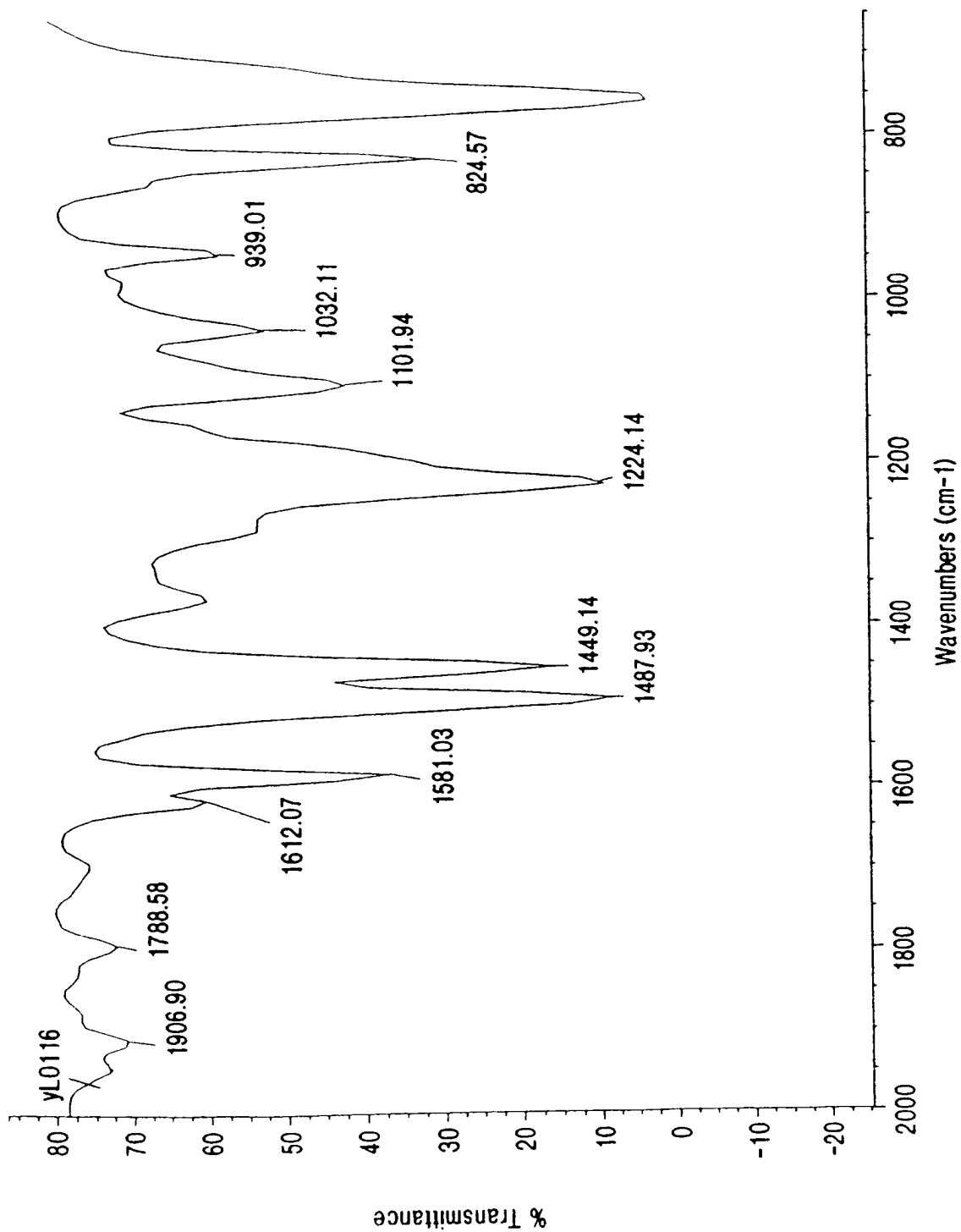
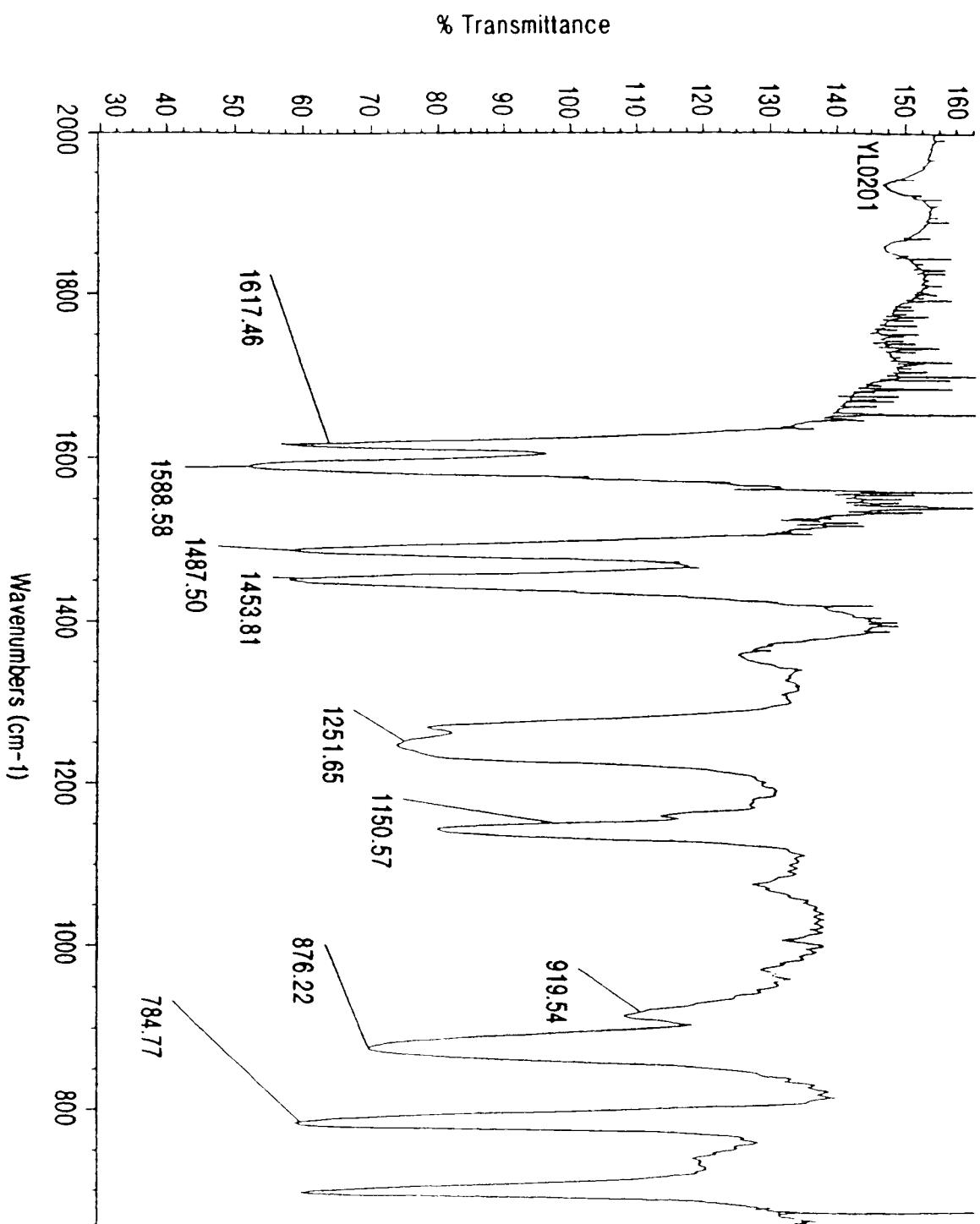


FIG. 63

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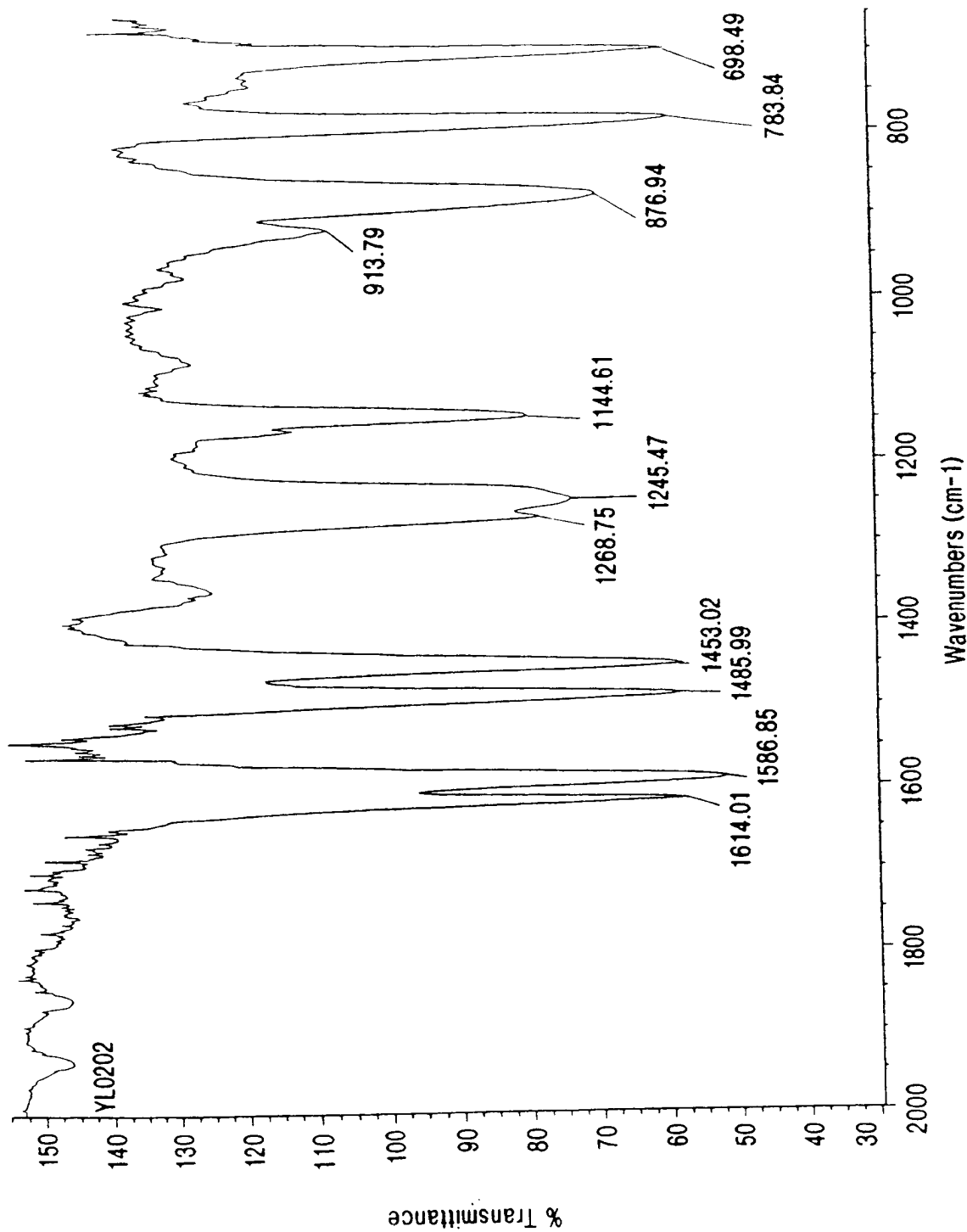
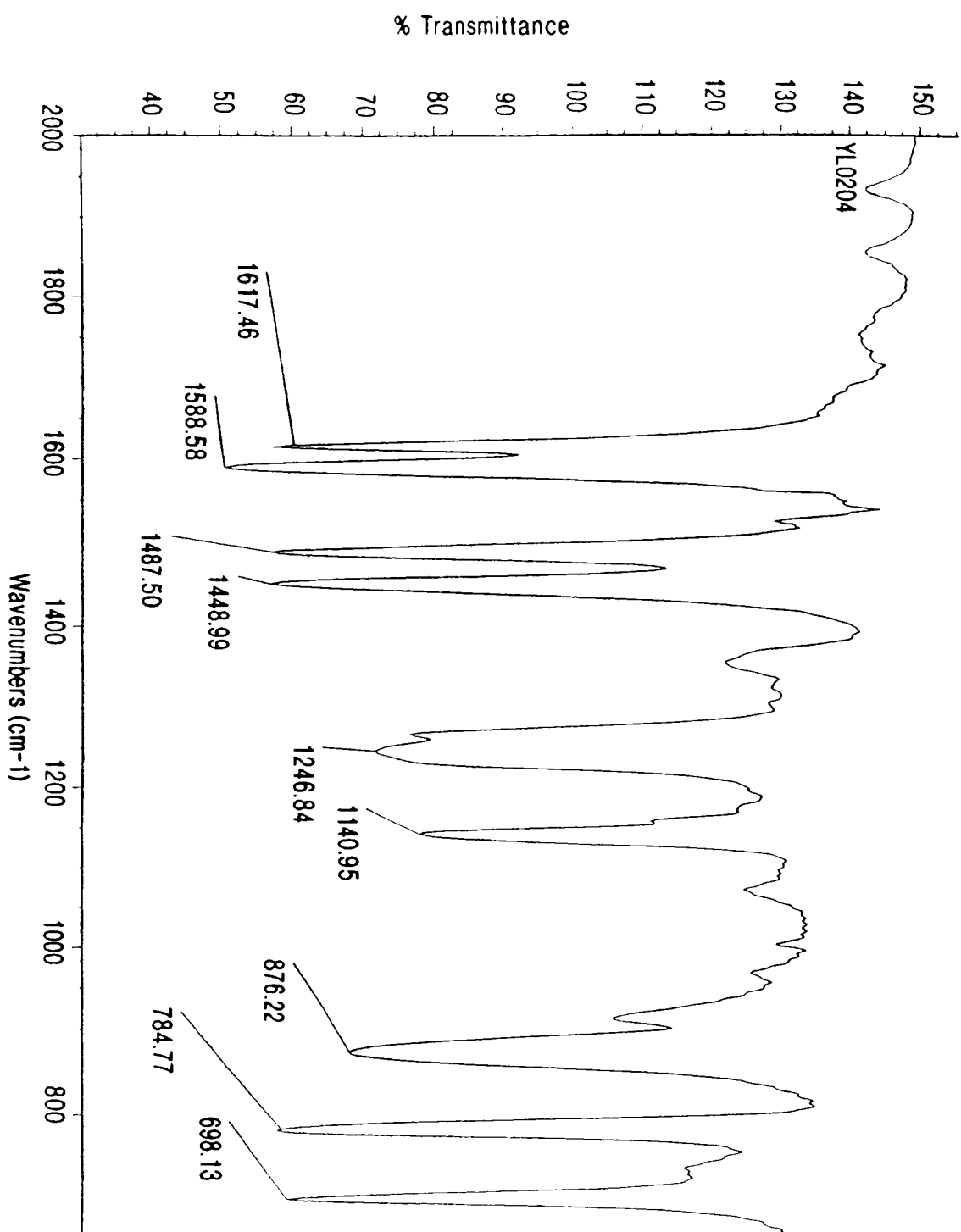


FIG. 65

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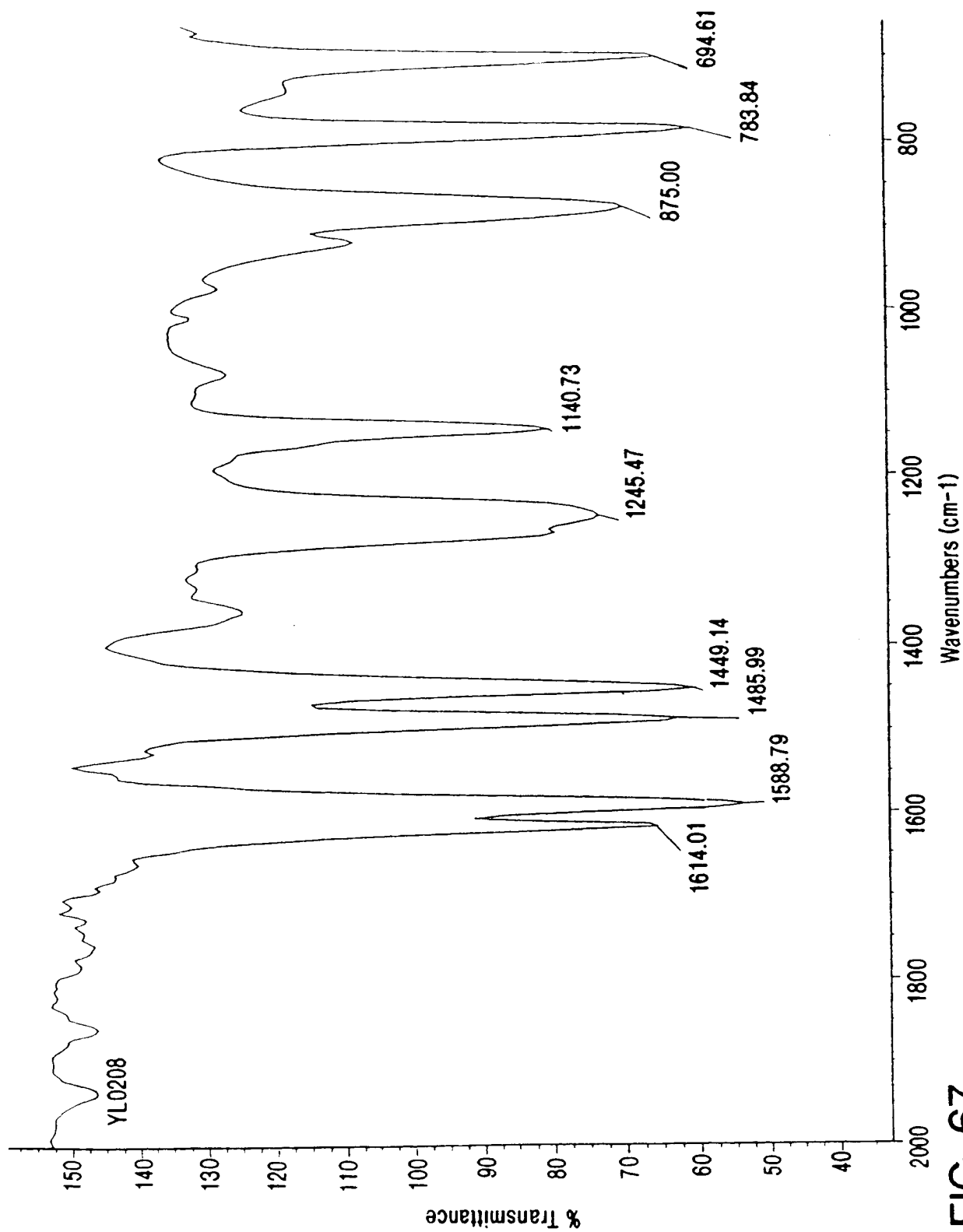
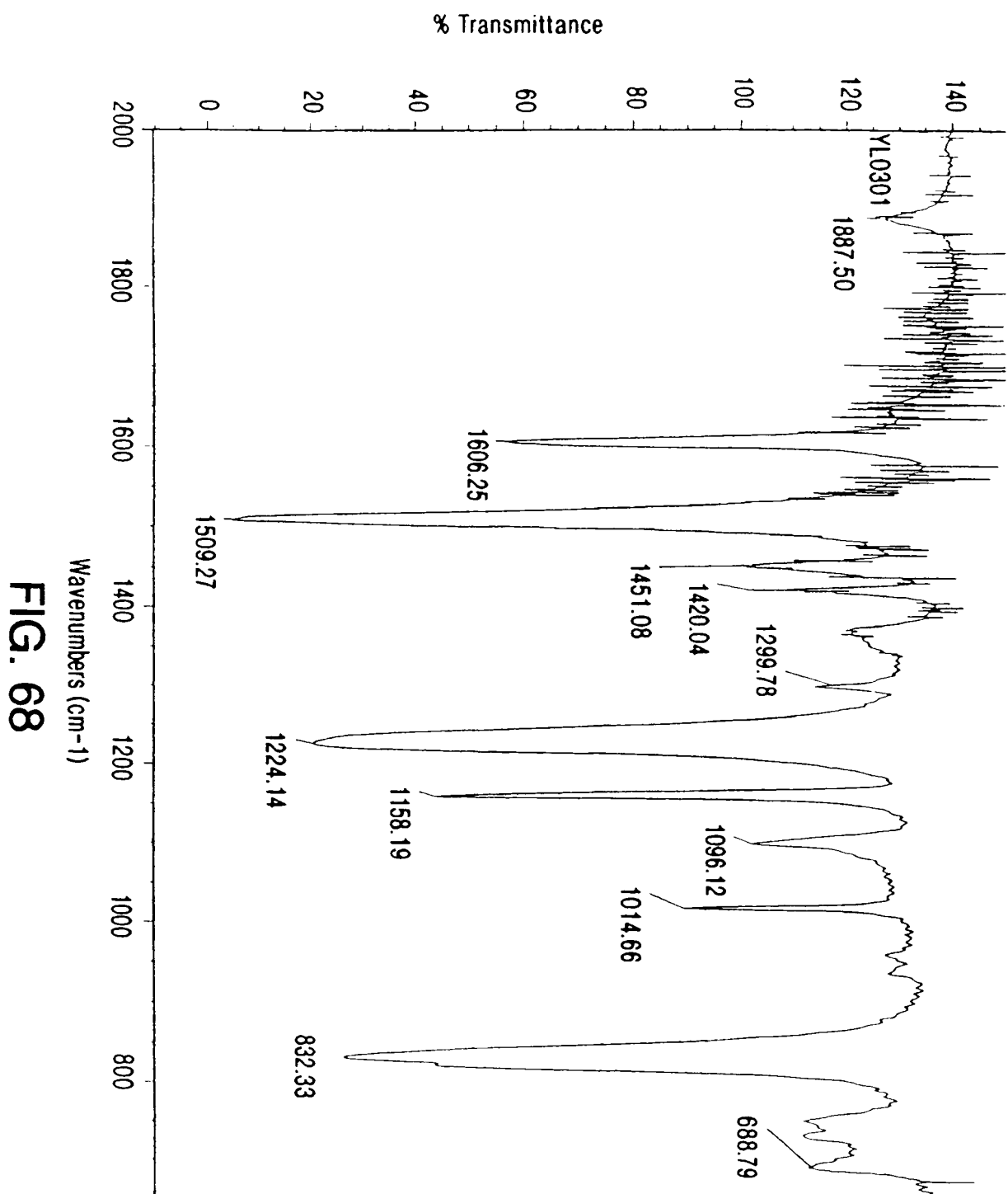


FIG. 67

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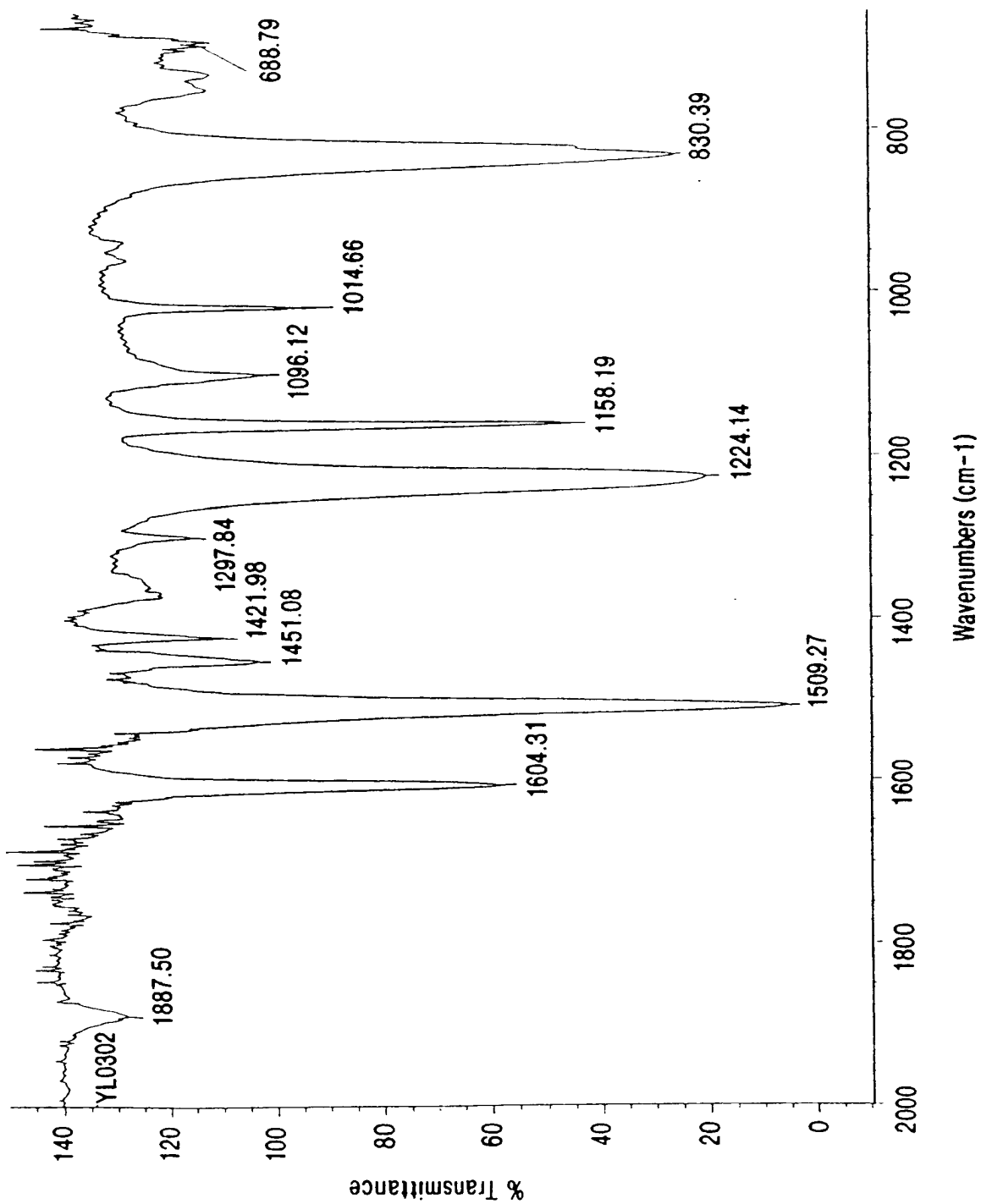


FIG. 69

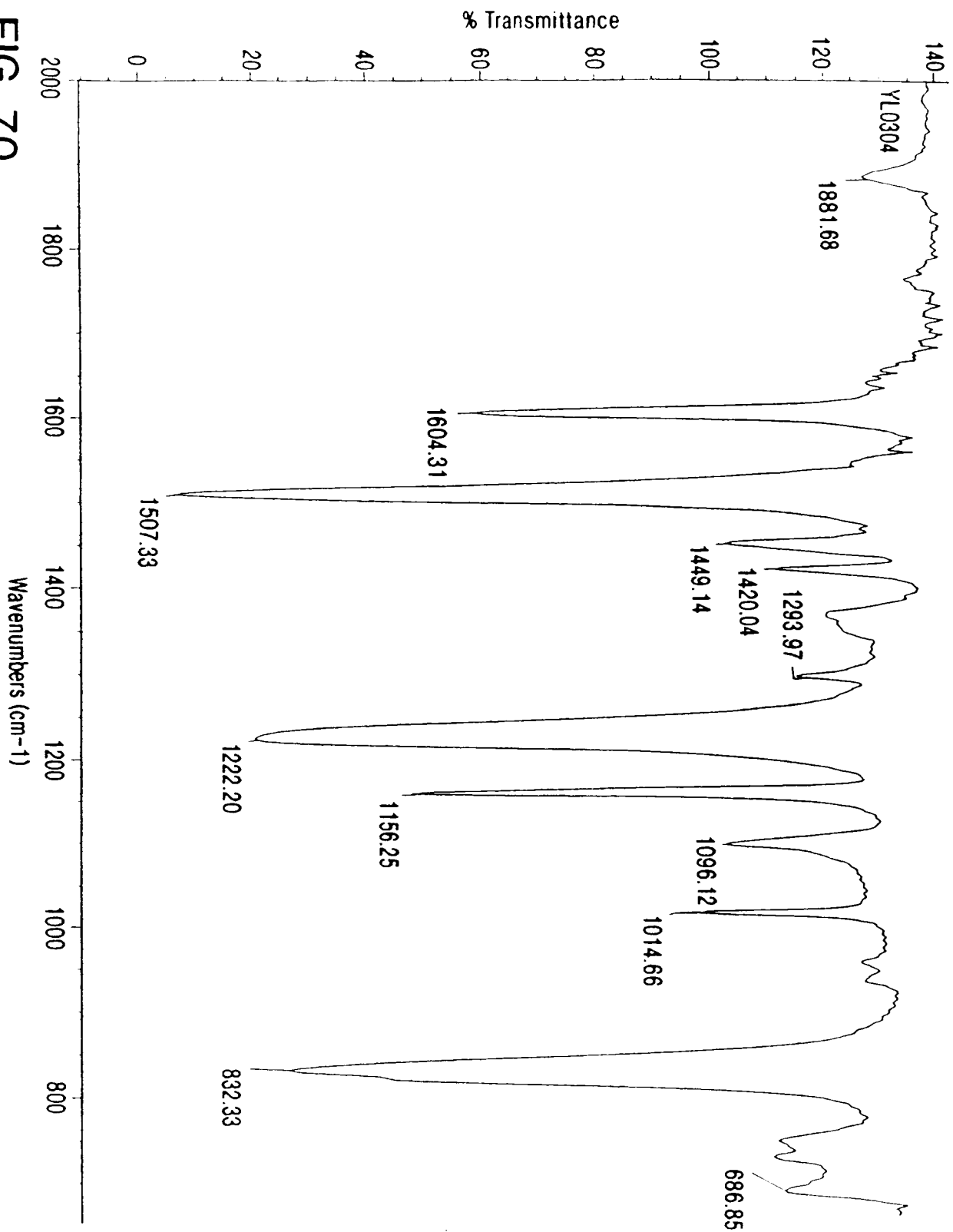


FIG. 70

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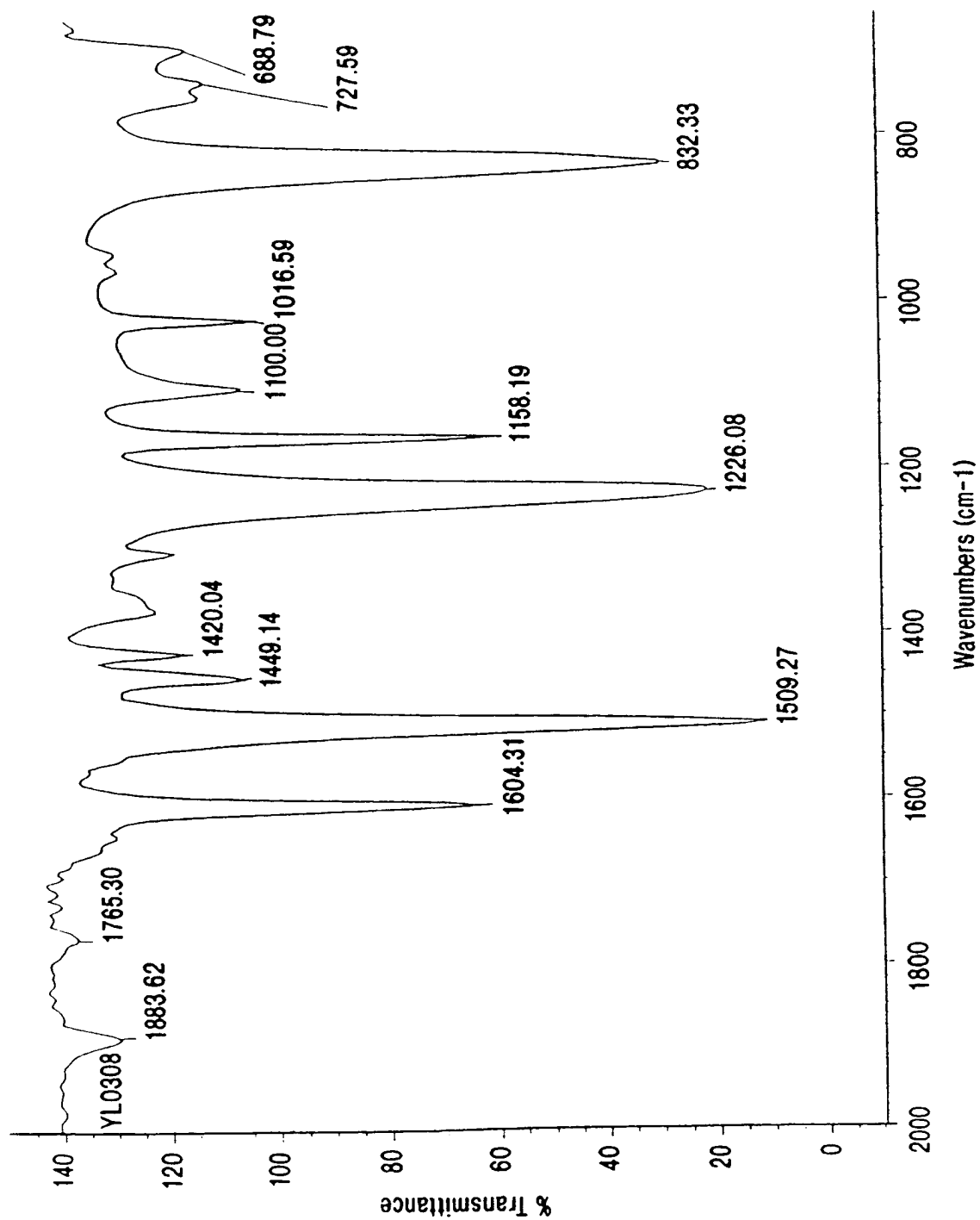
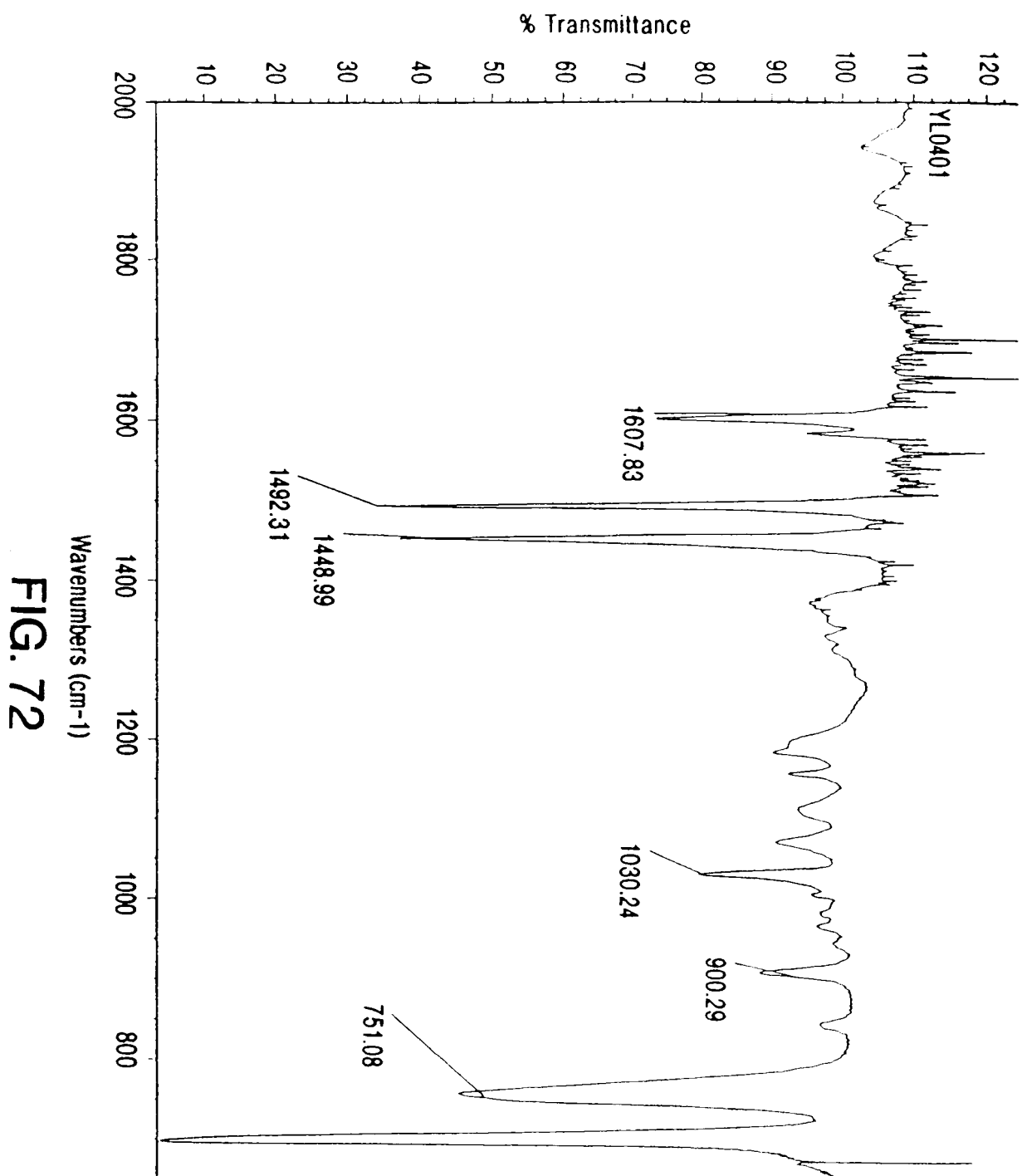


FIG. 71

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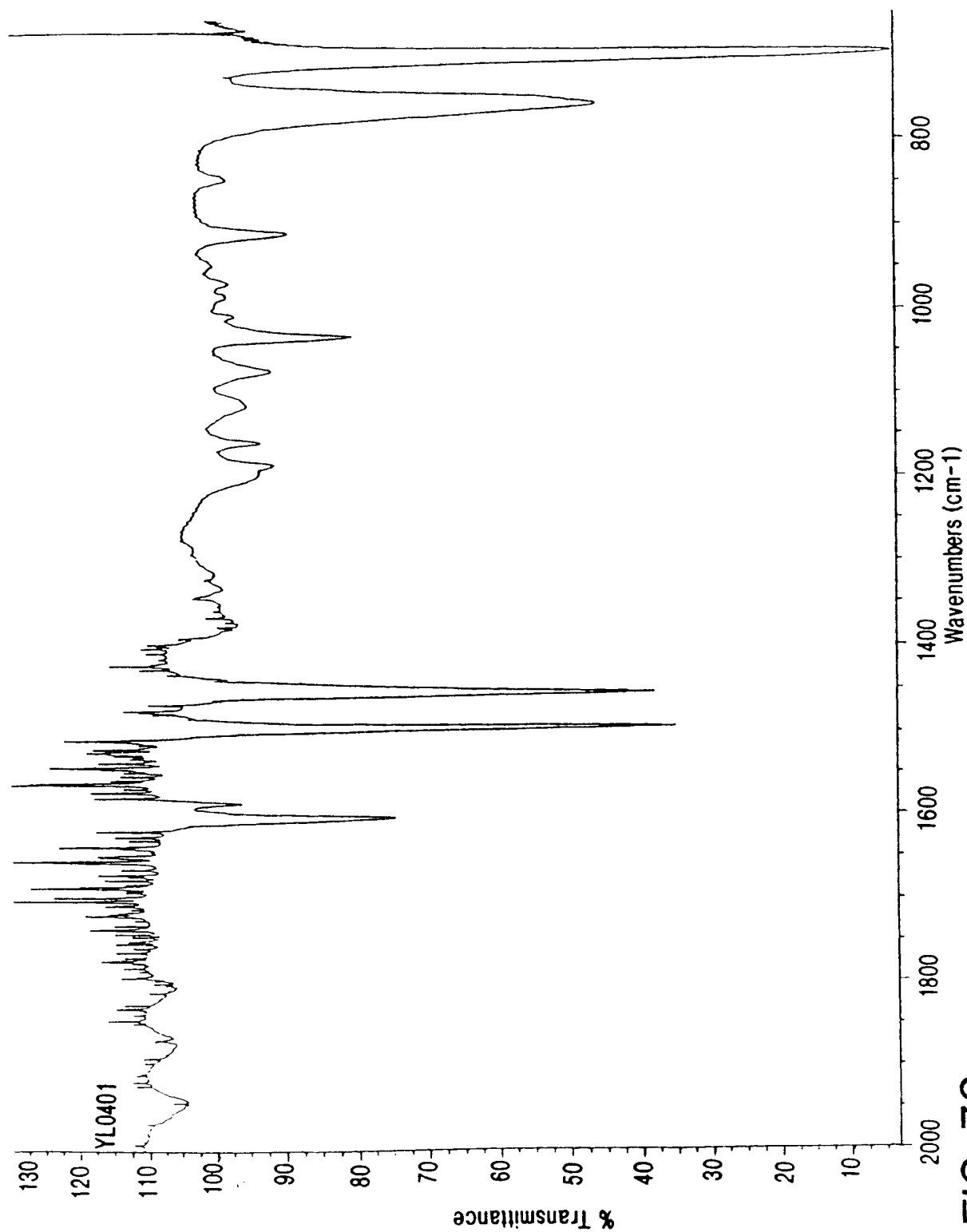


FIG. 73

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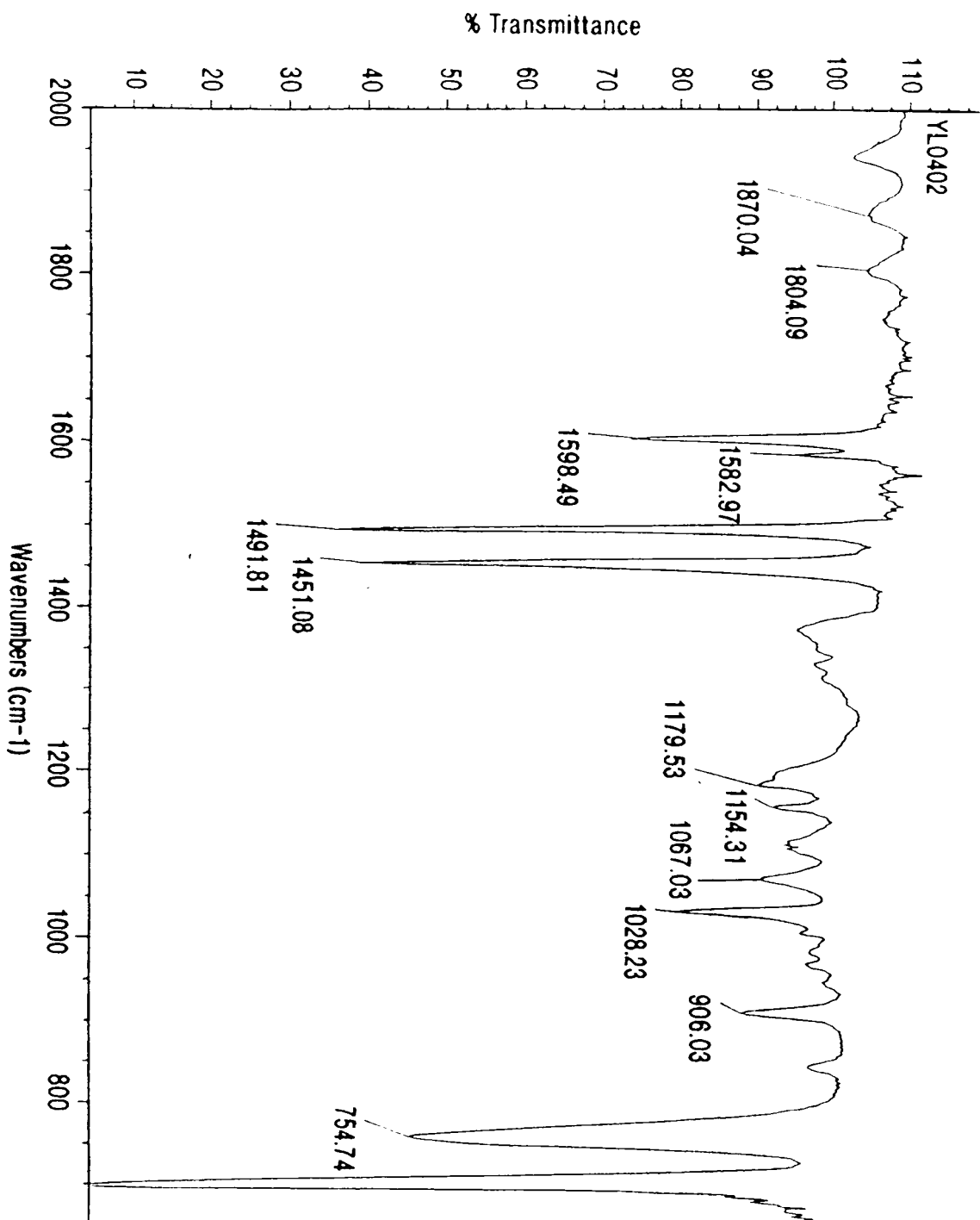


FIG. 74

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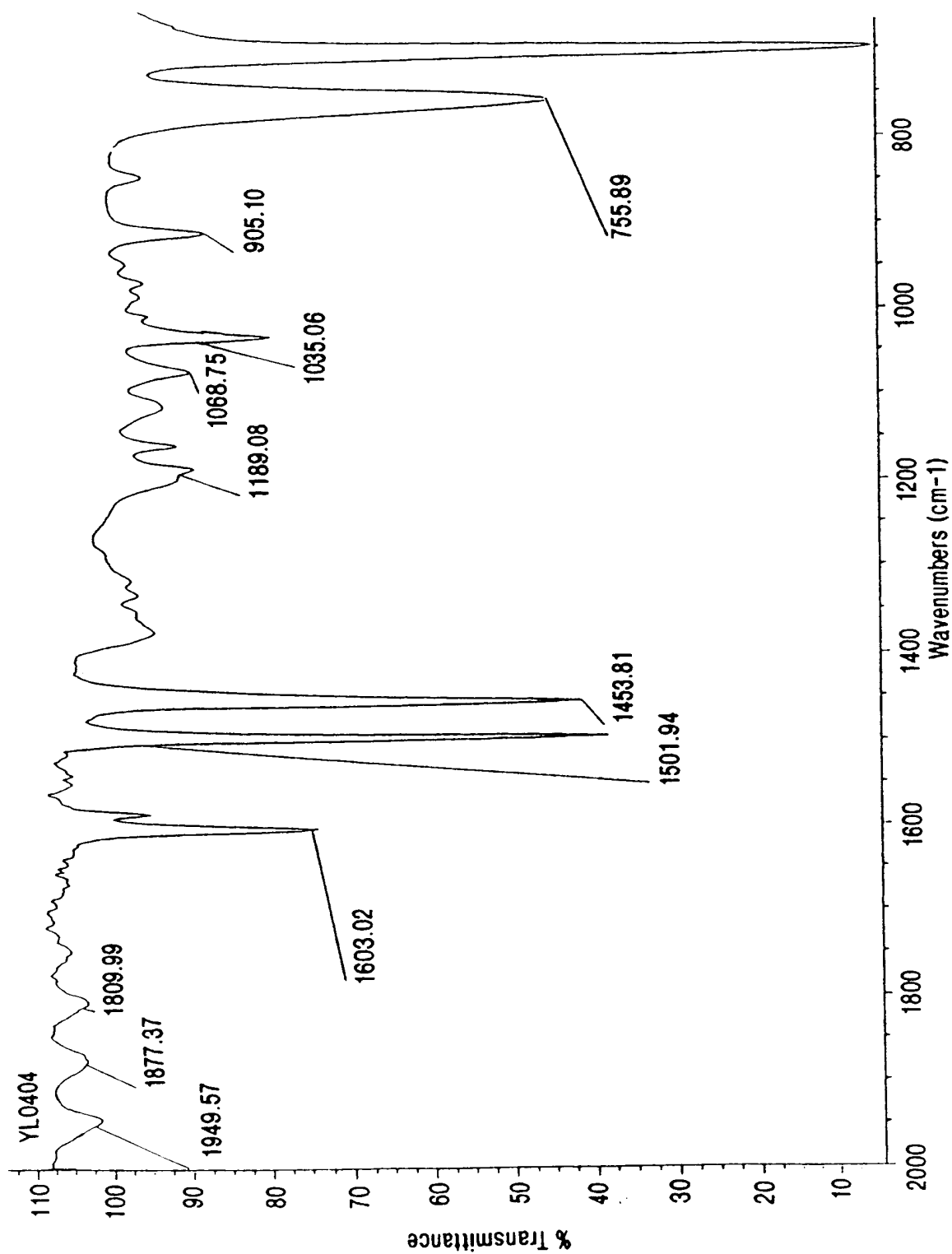


FIG. 75

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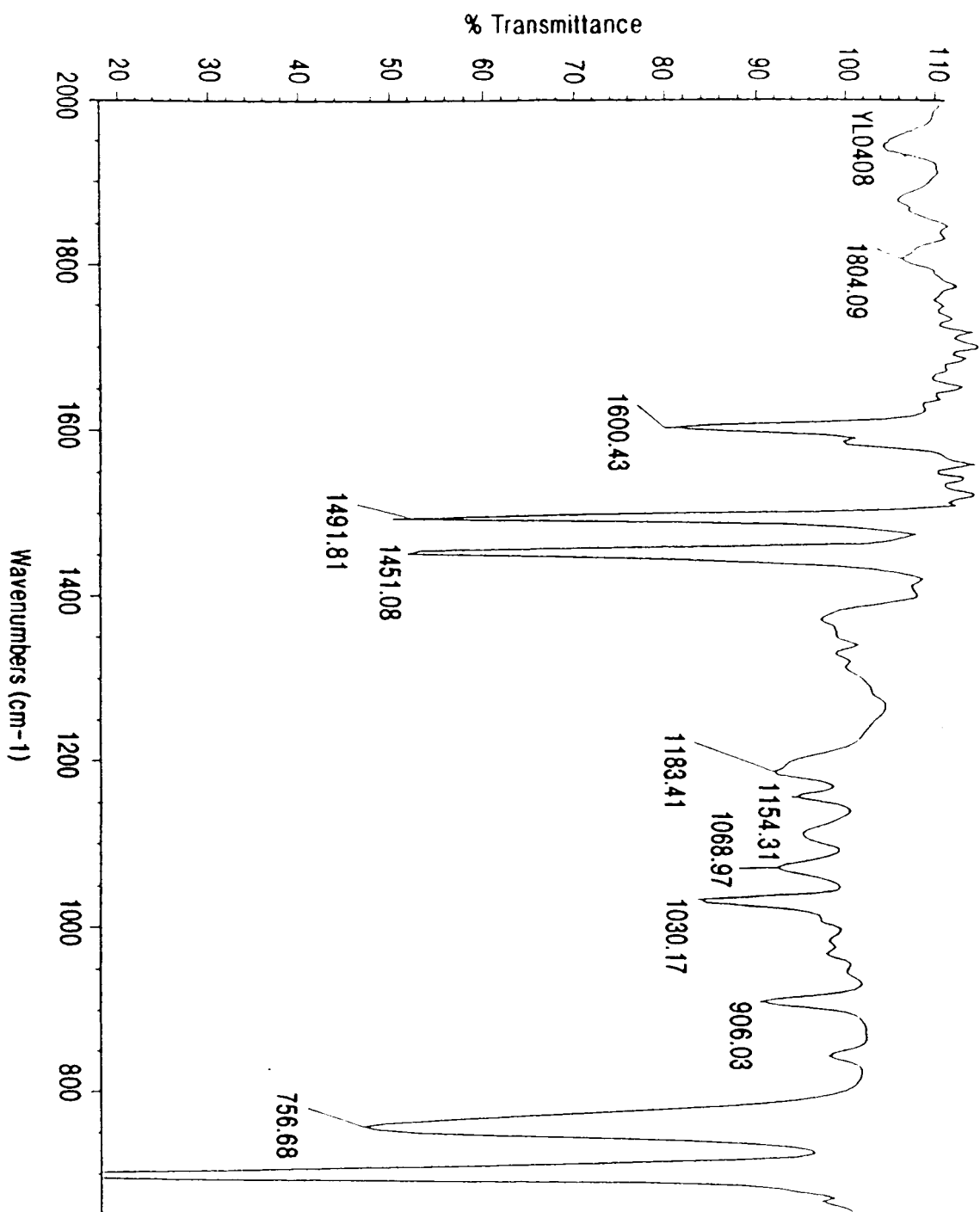


FIG. 76

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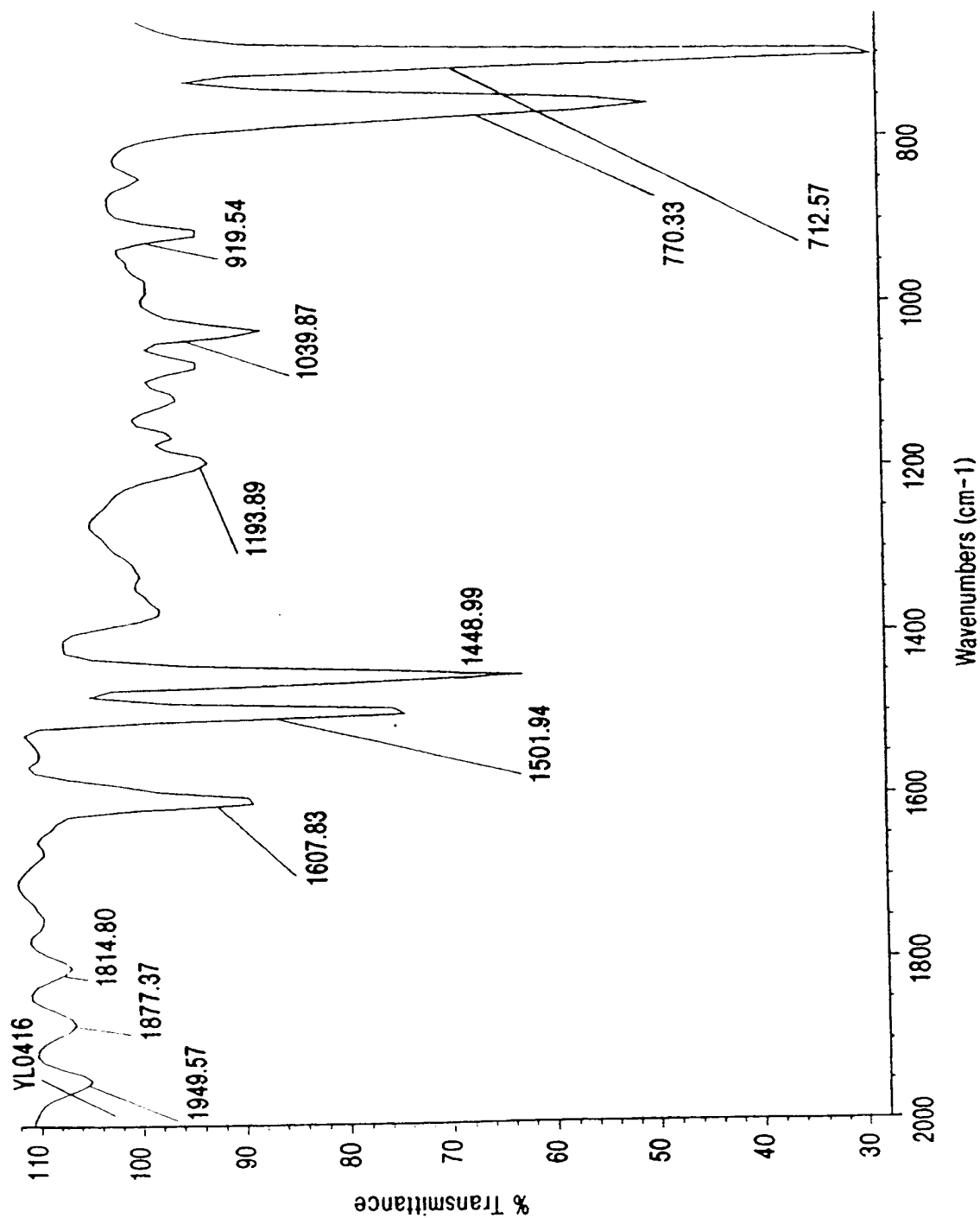


FIG. 77

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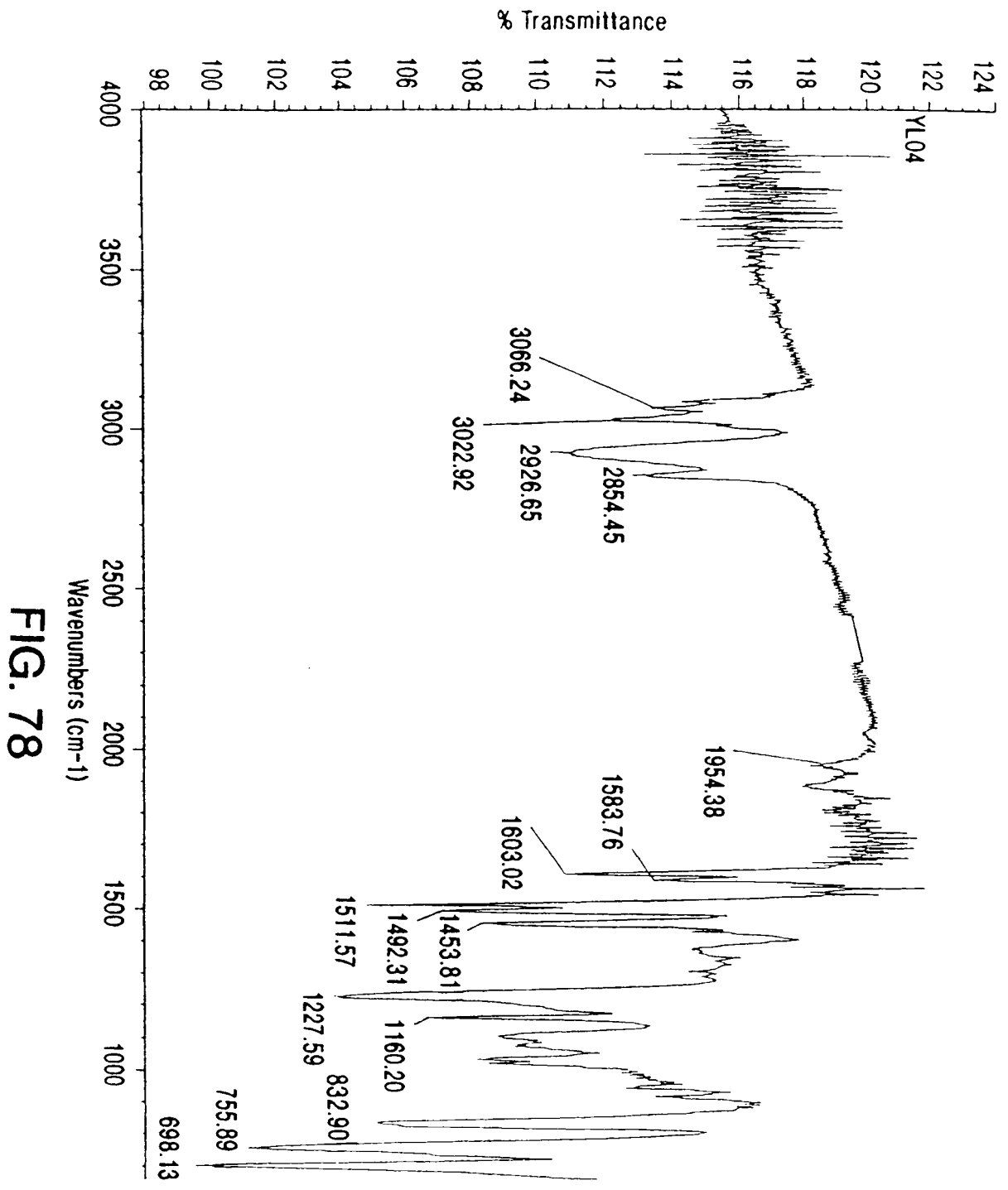


FIG. 78

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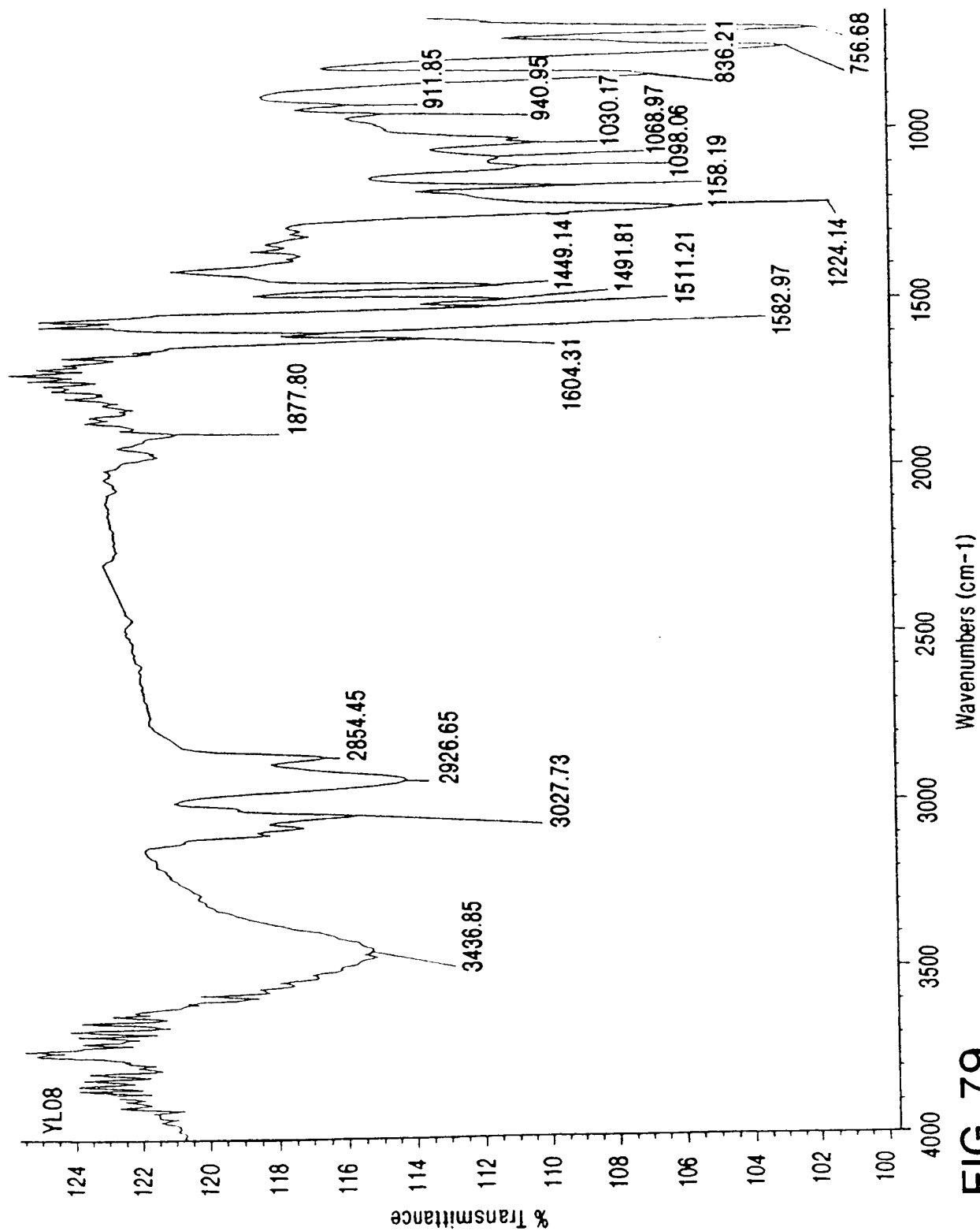
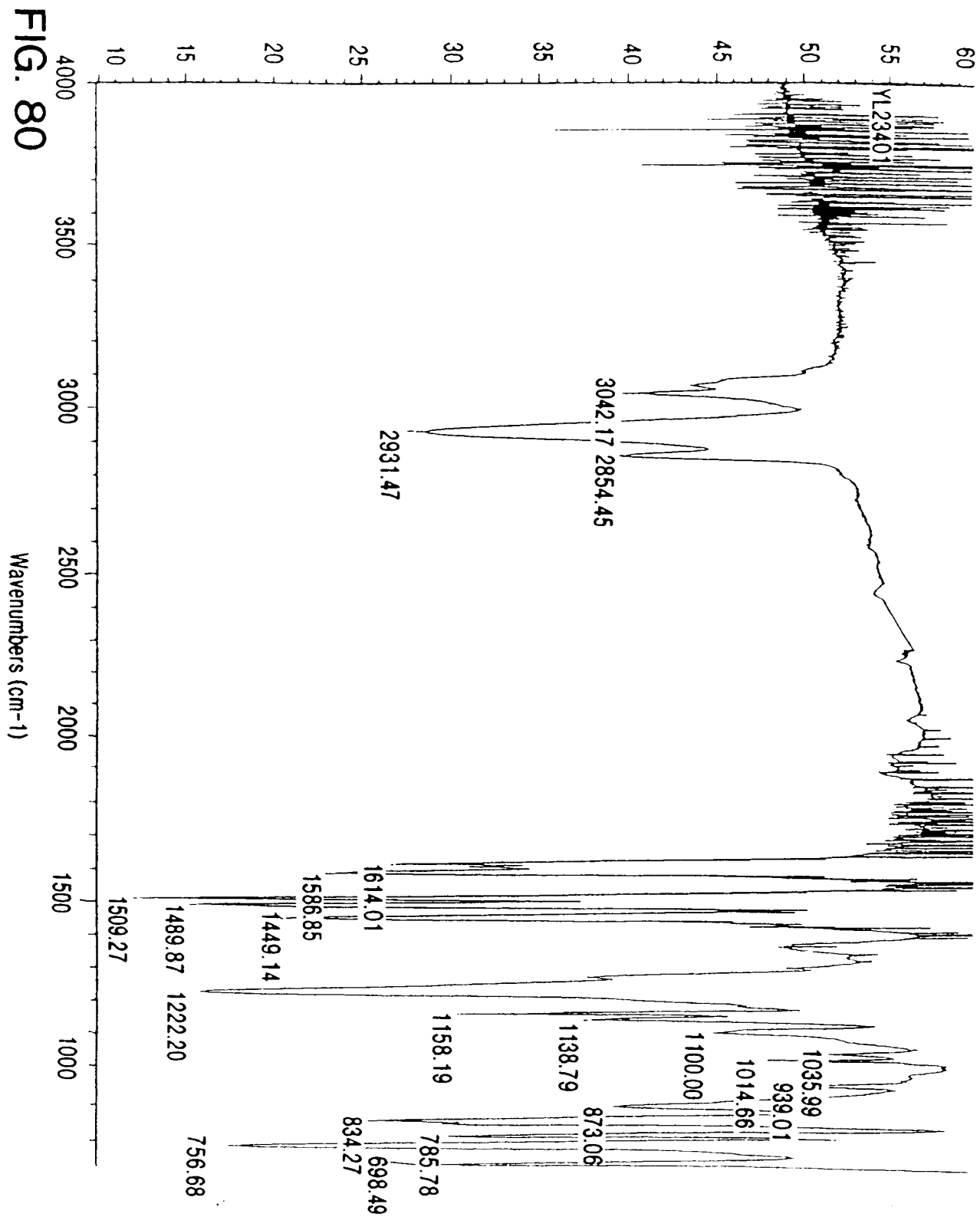


FIG. 79

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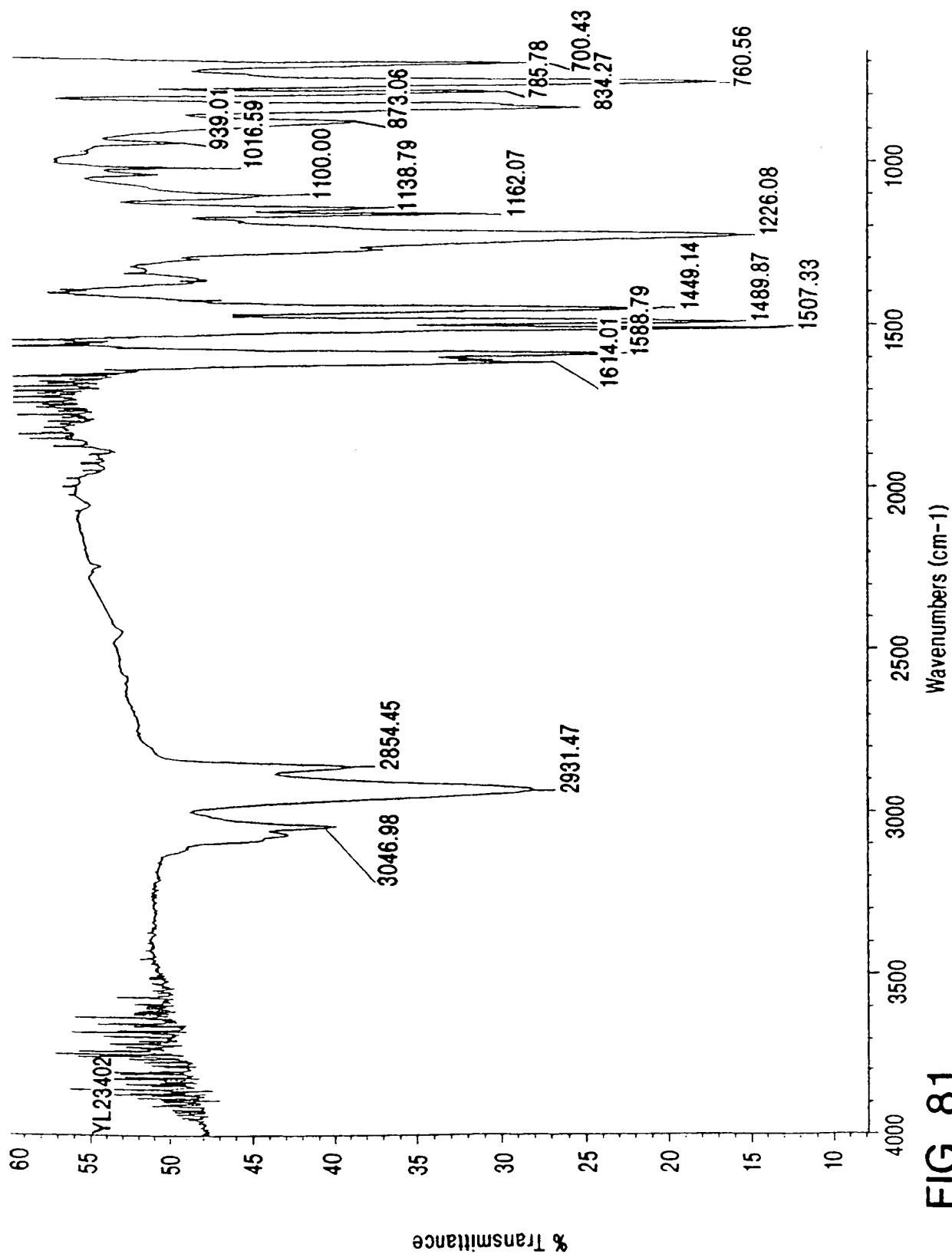


FIG. 81

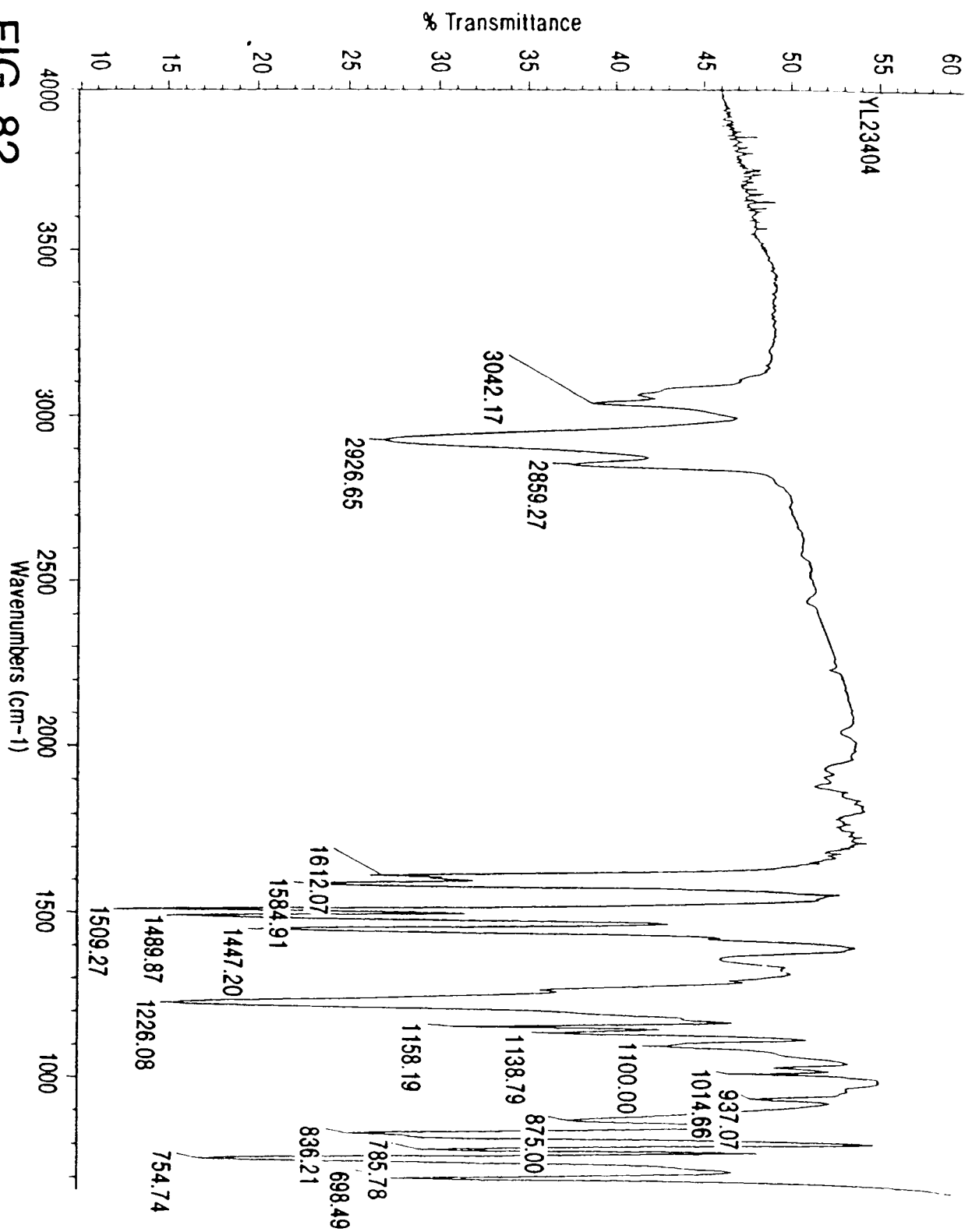


FIG. 82

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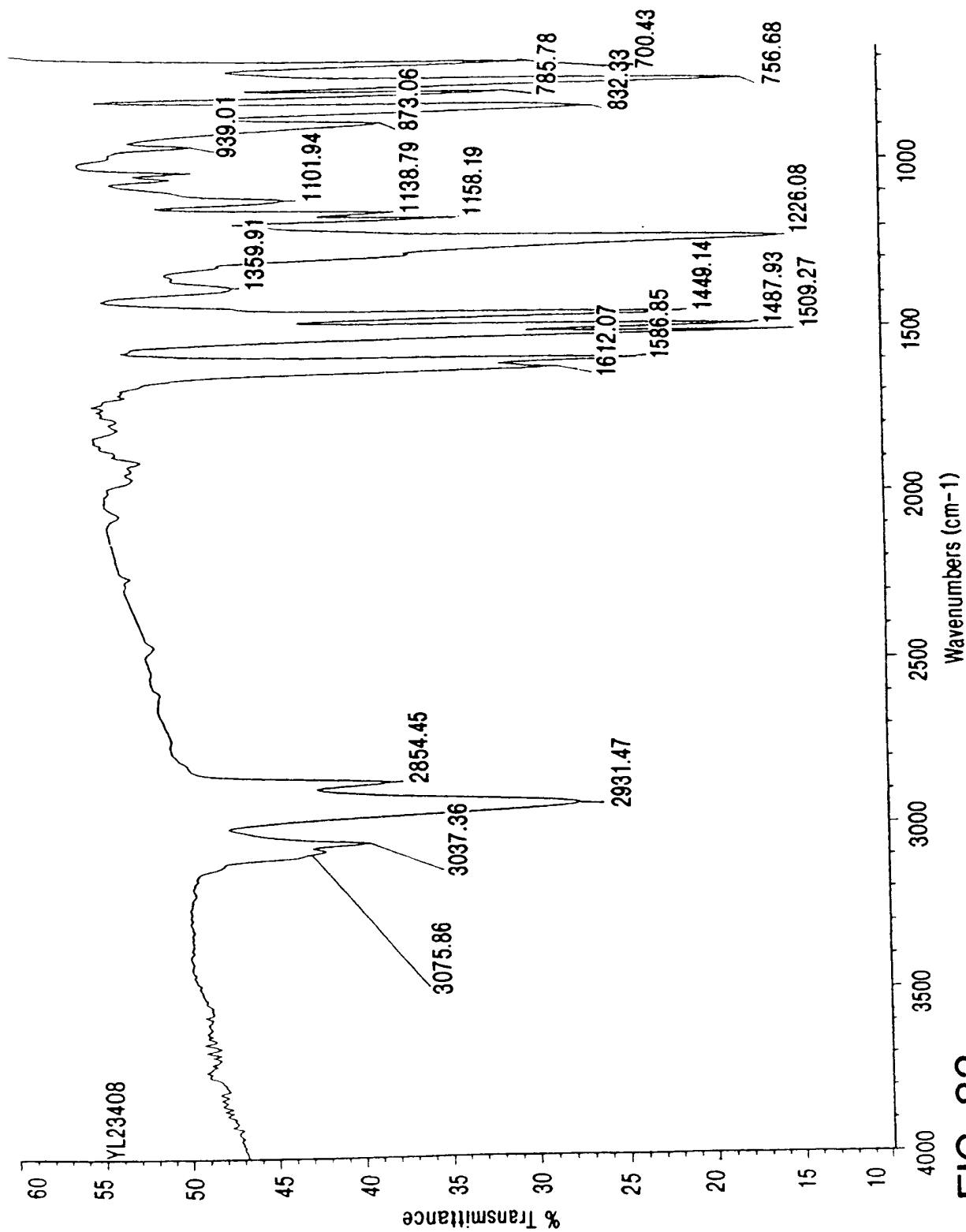
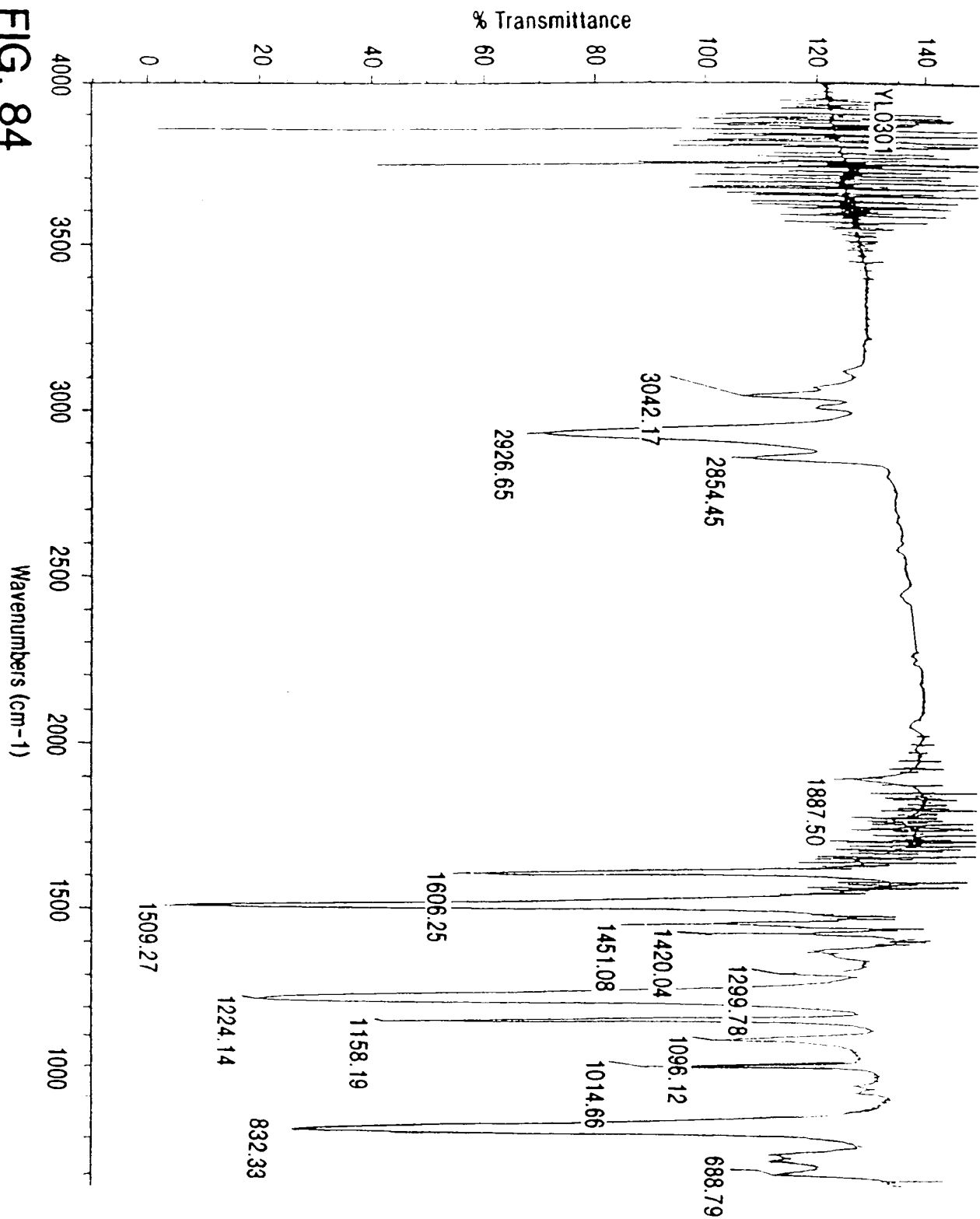


FIG. 83

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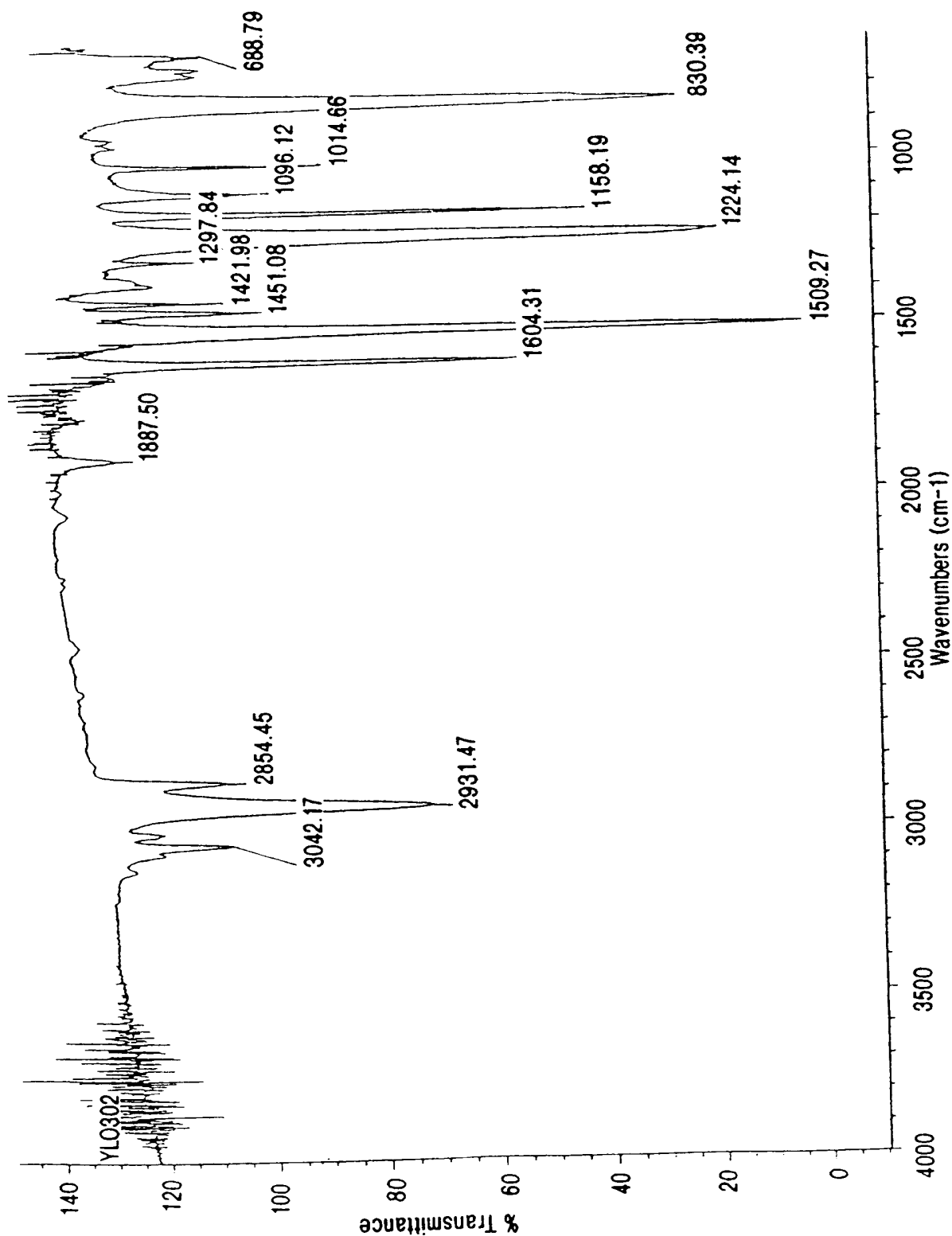
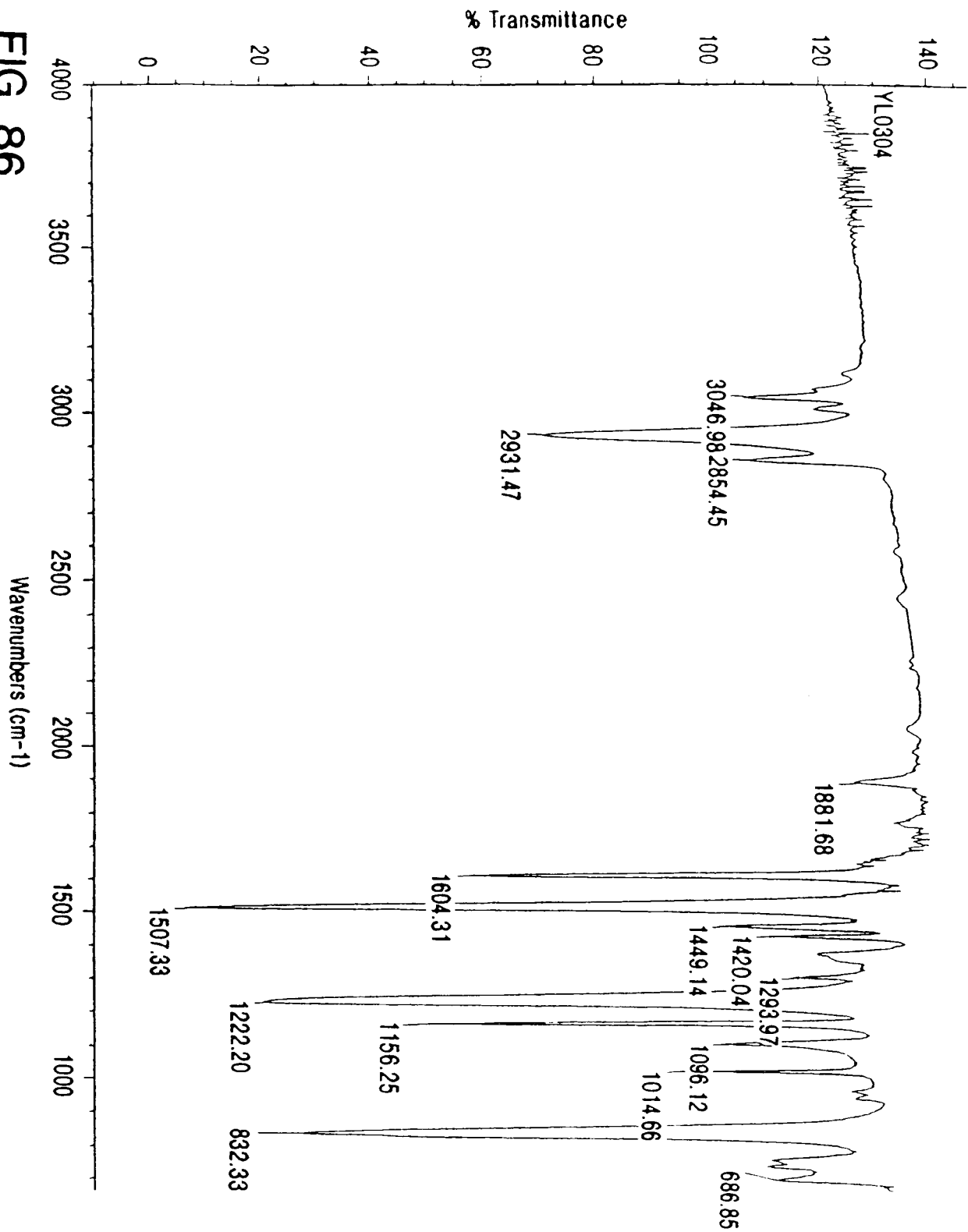


FIG. 85

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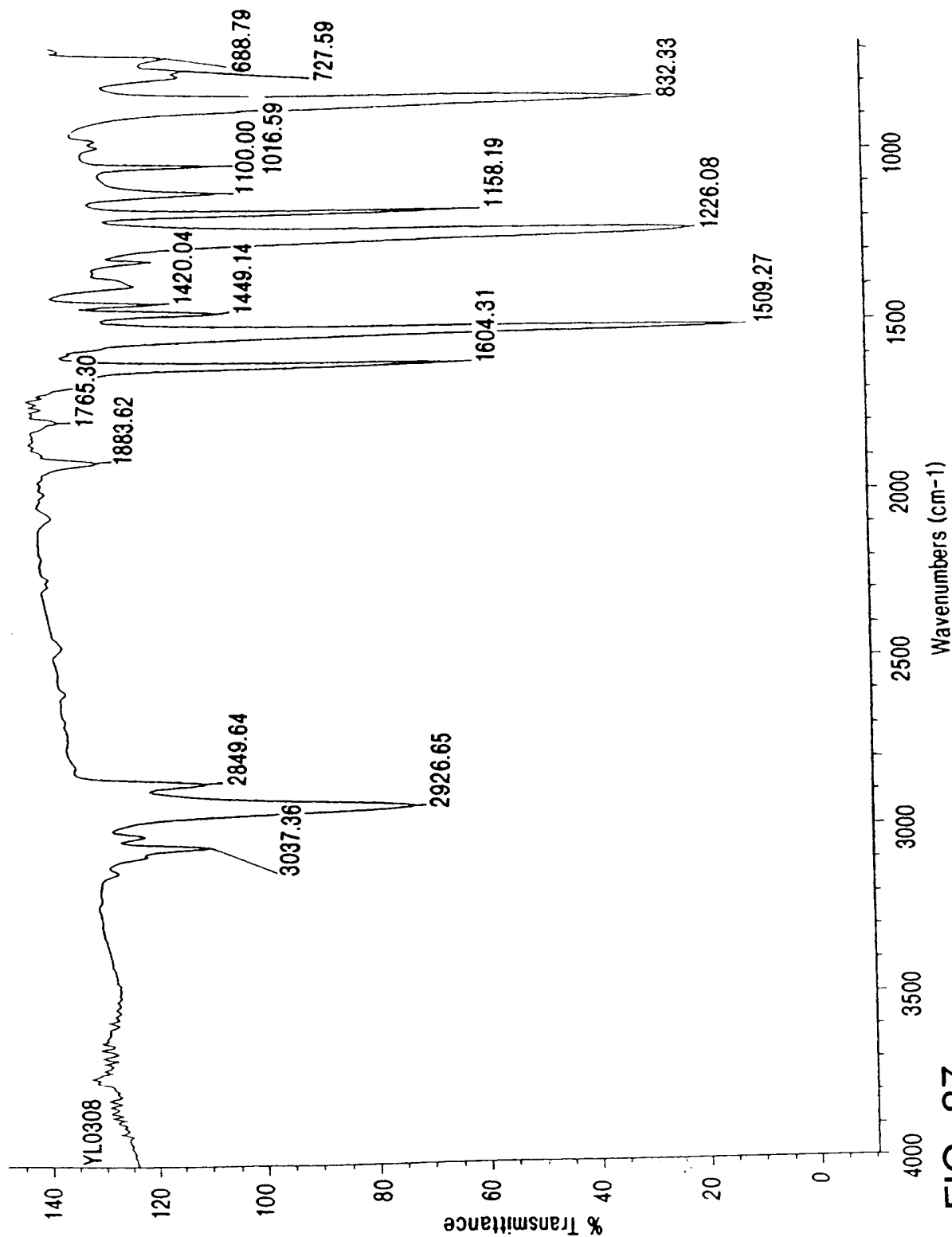


FIG. 87

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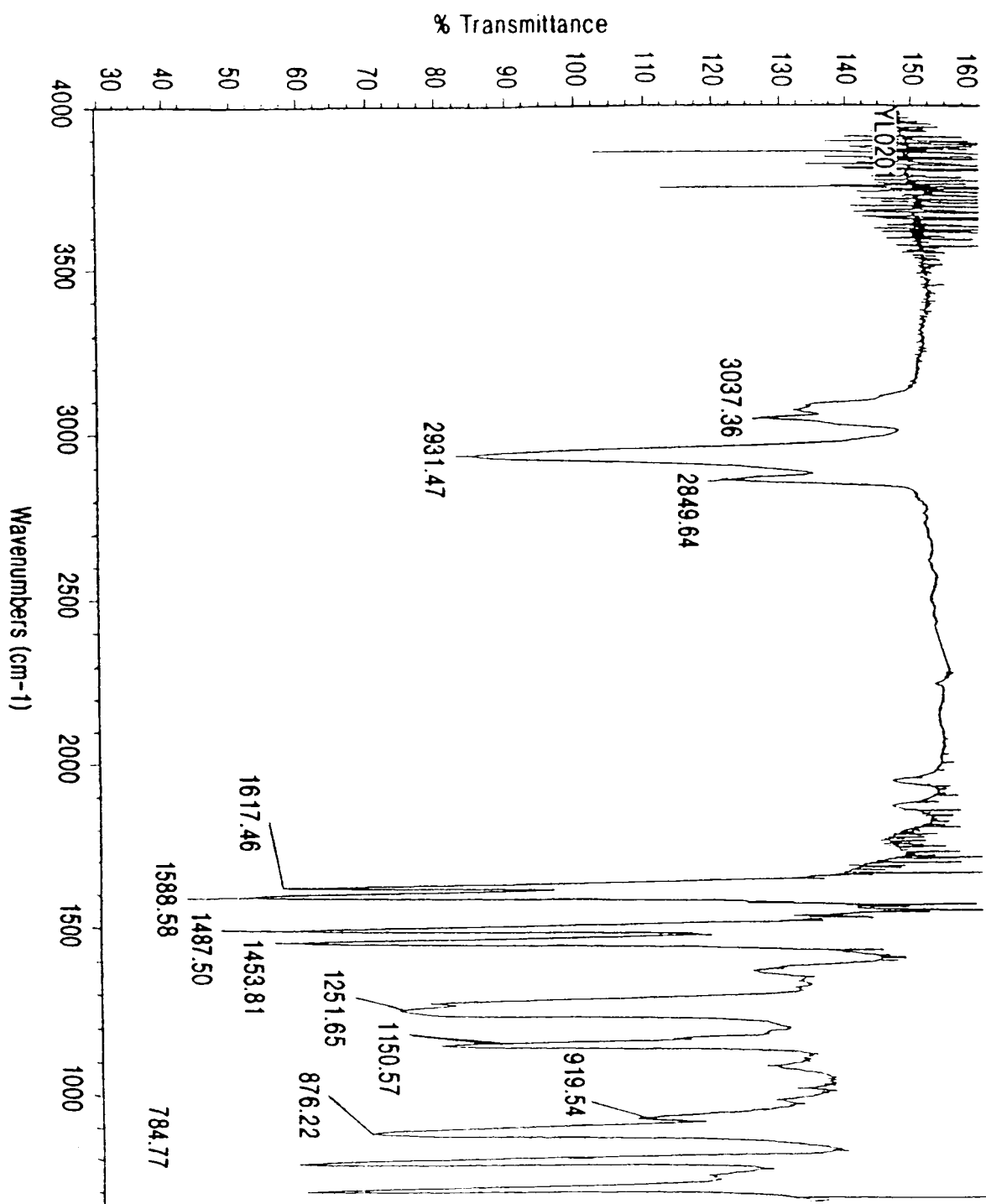


FIG. 88

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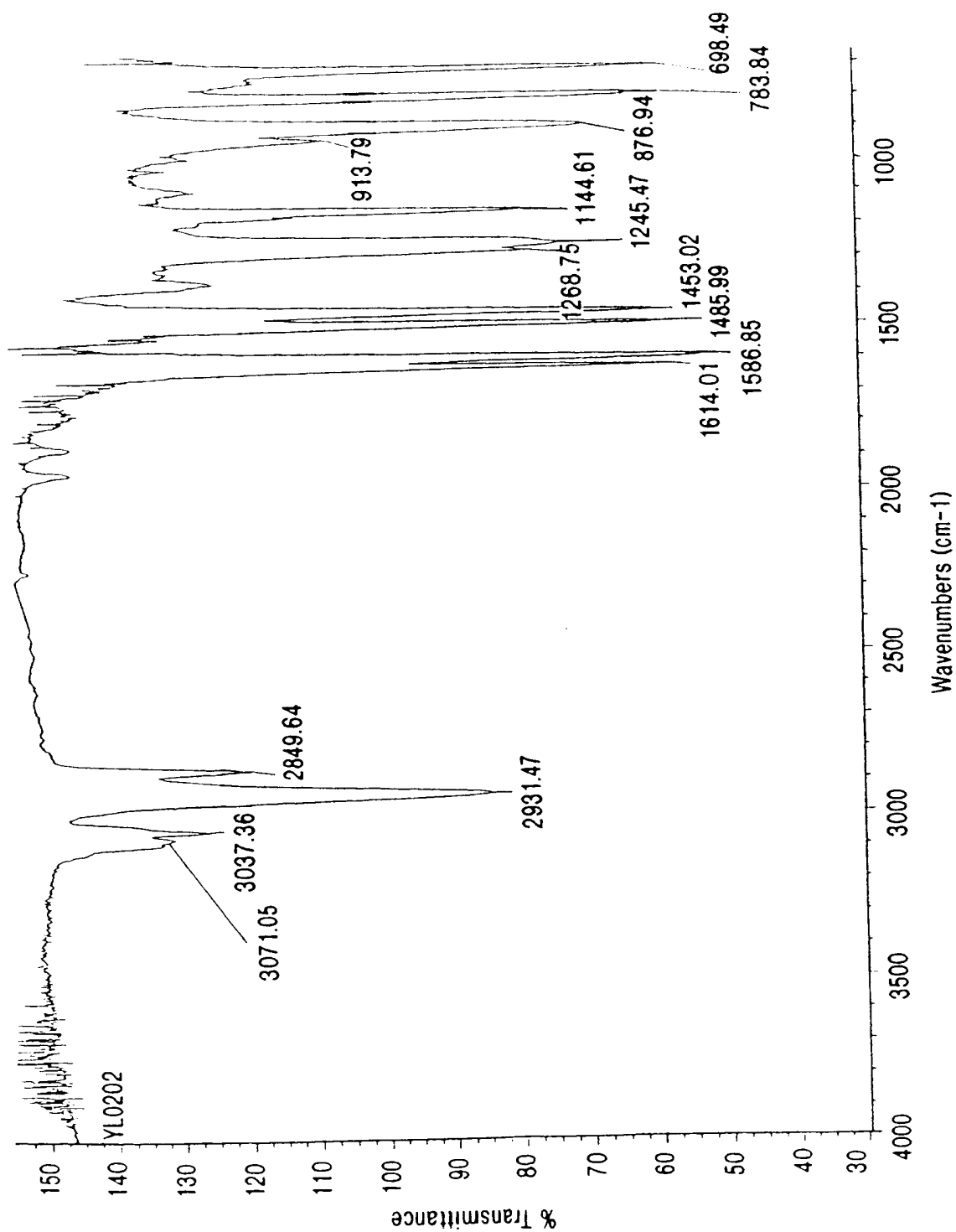
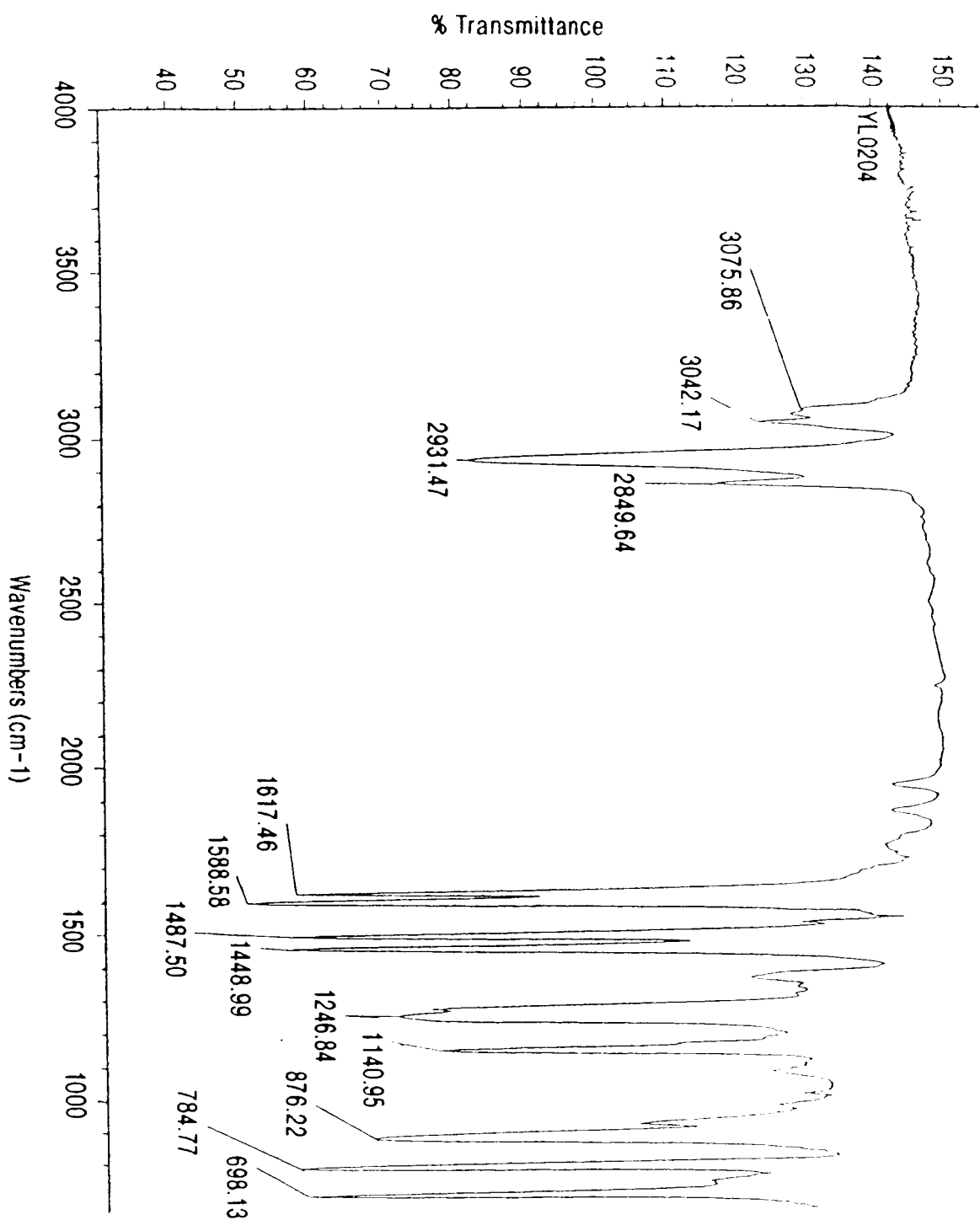


FIG. 89

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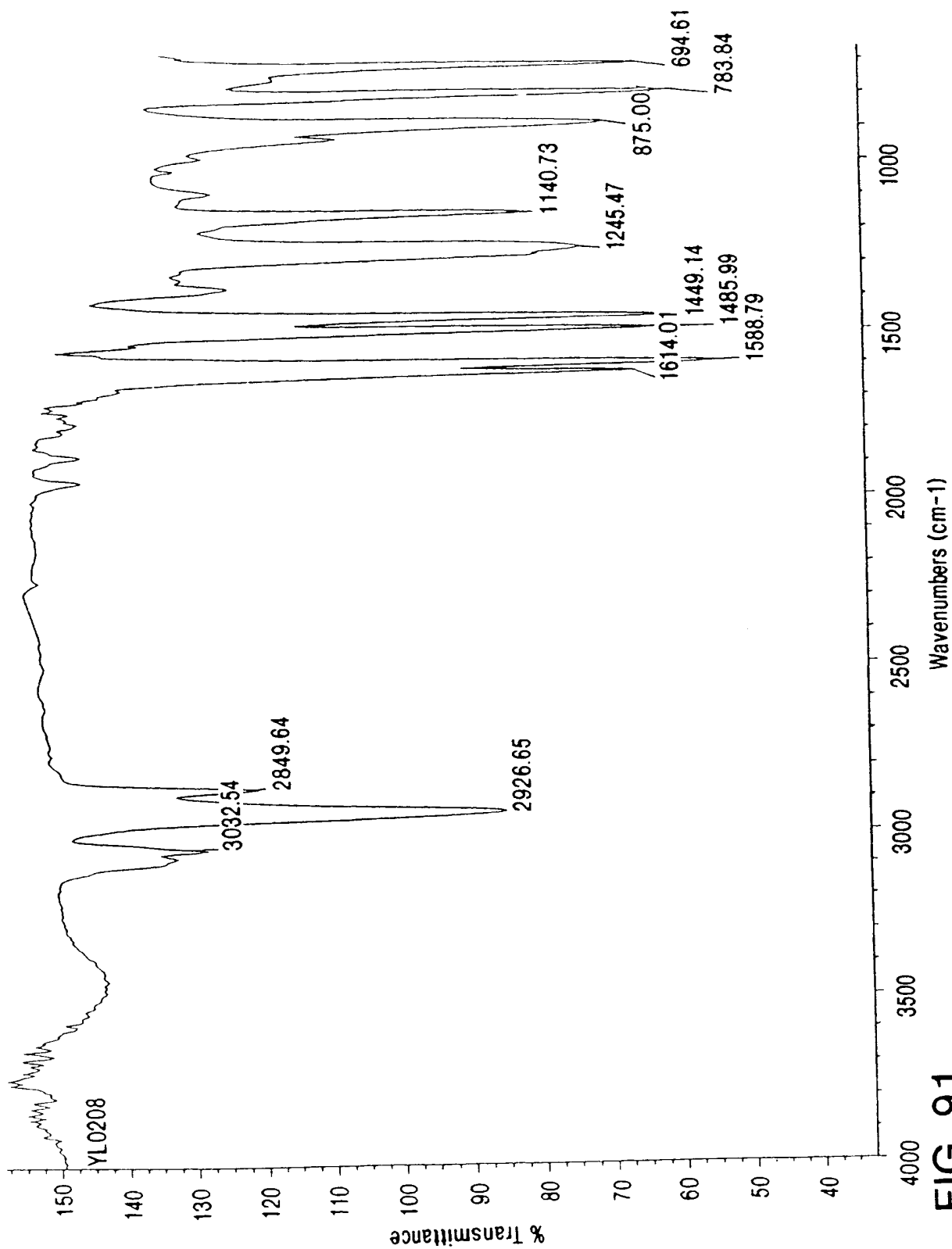
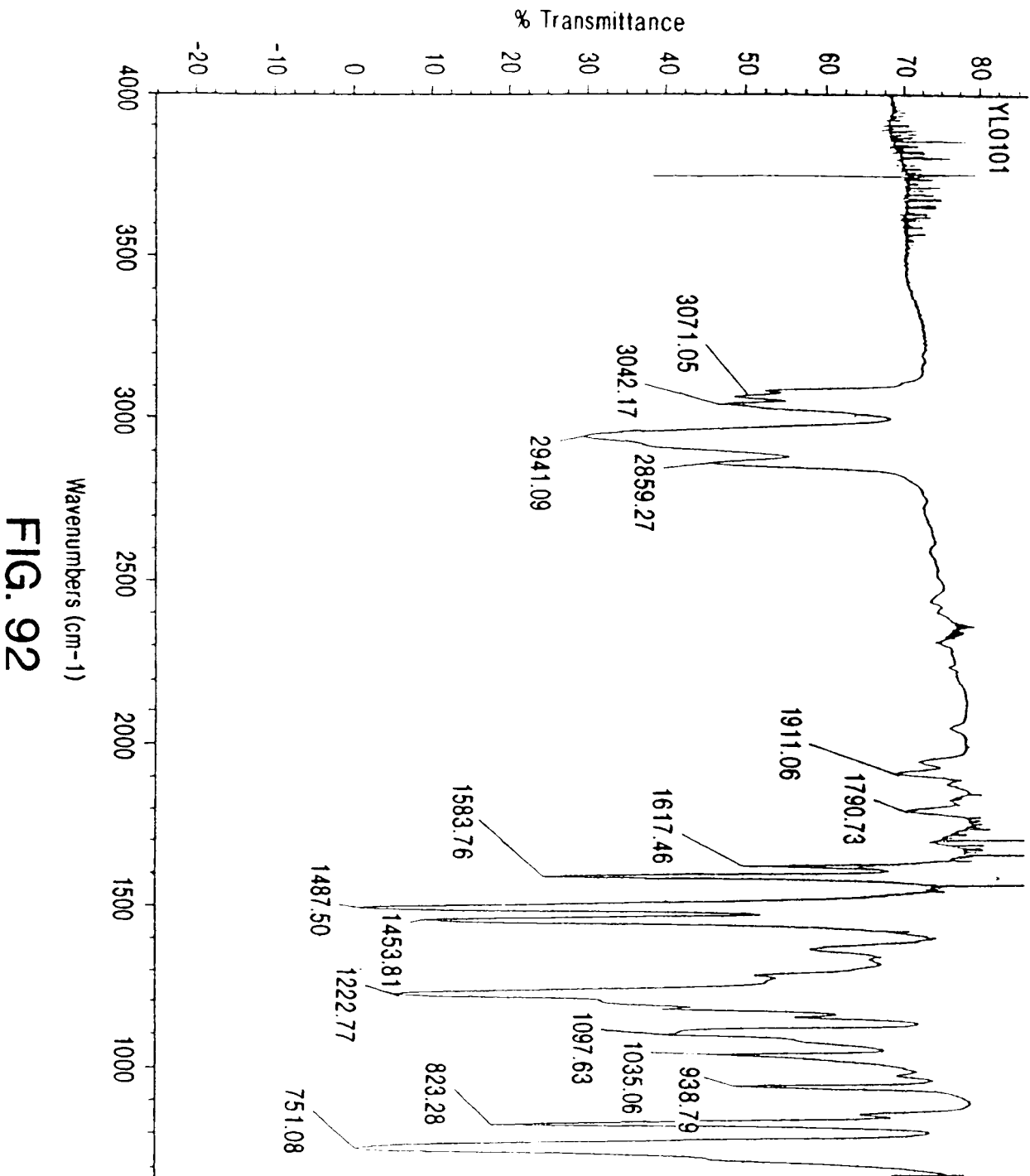


FIG. 91

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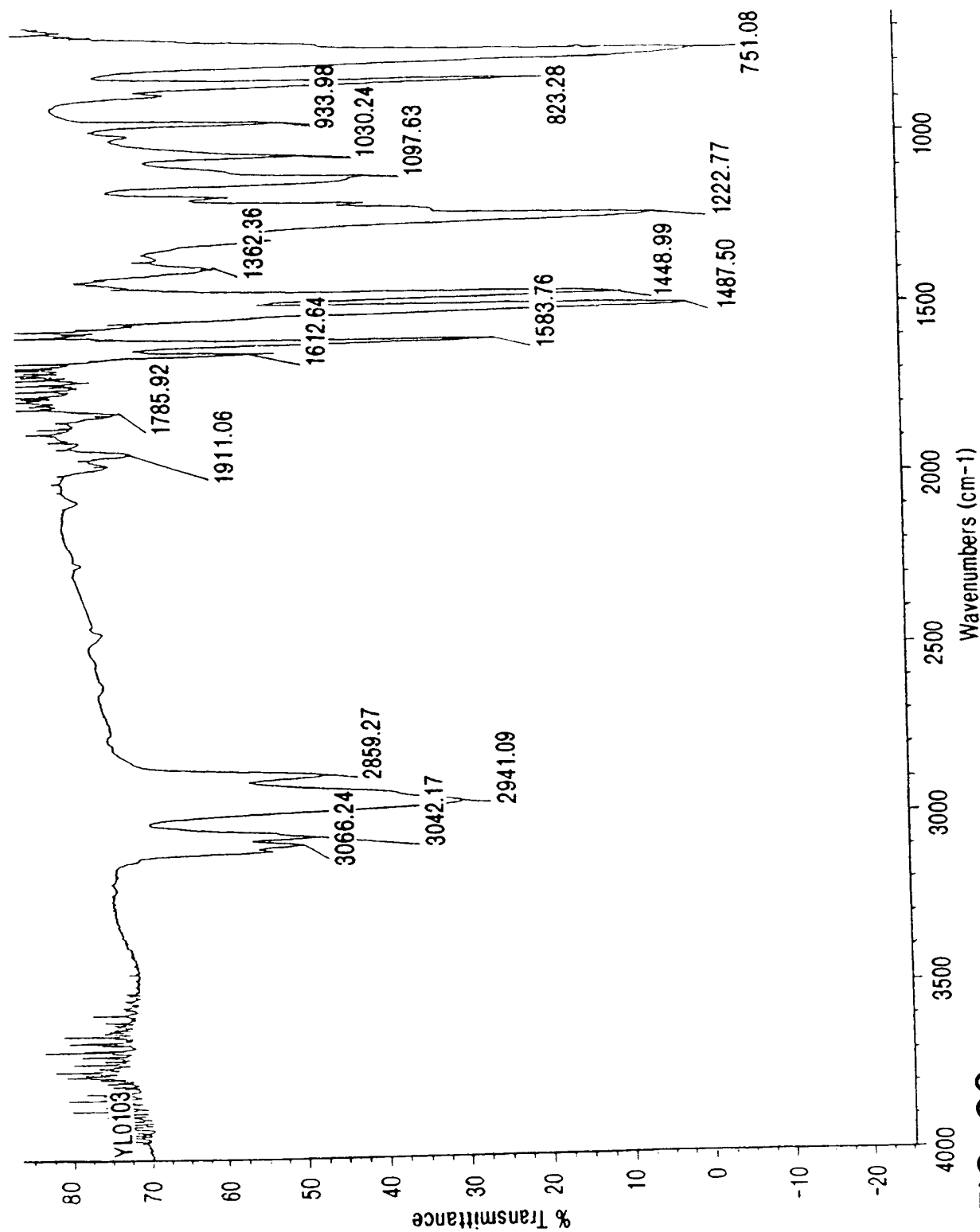


FIG. 93

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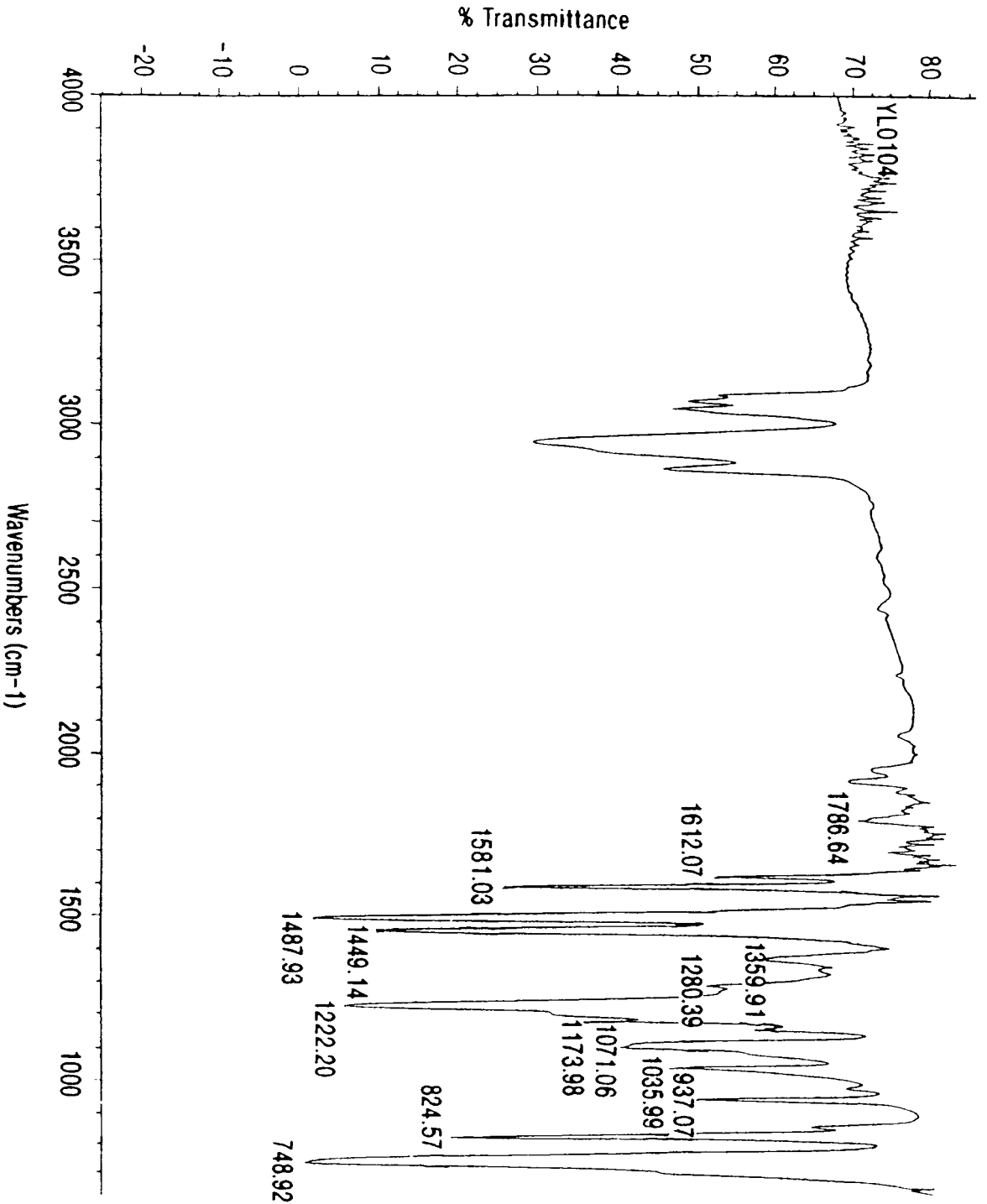


FIG. 94

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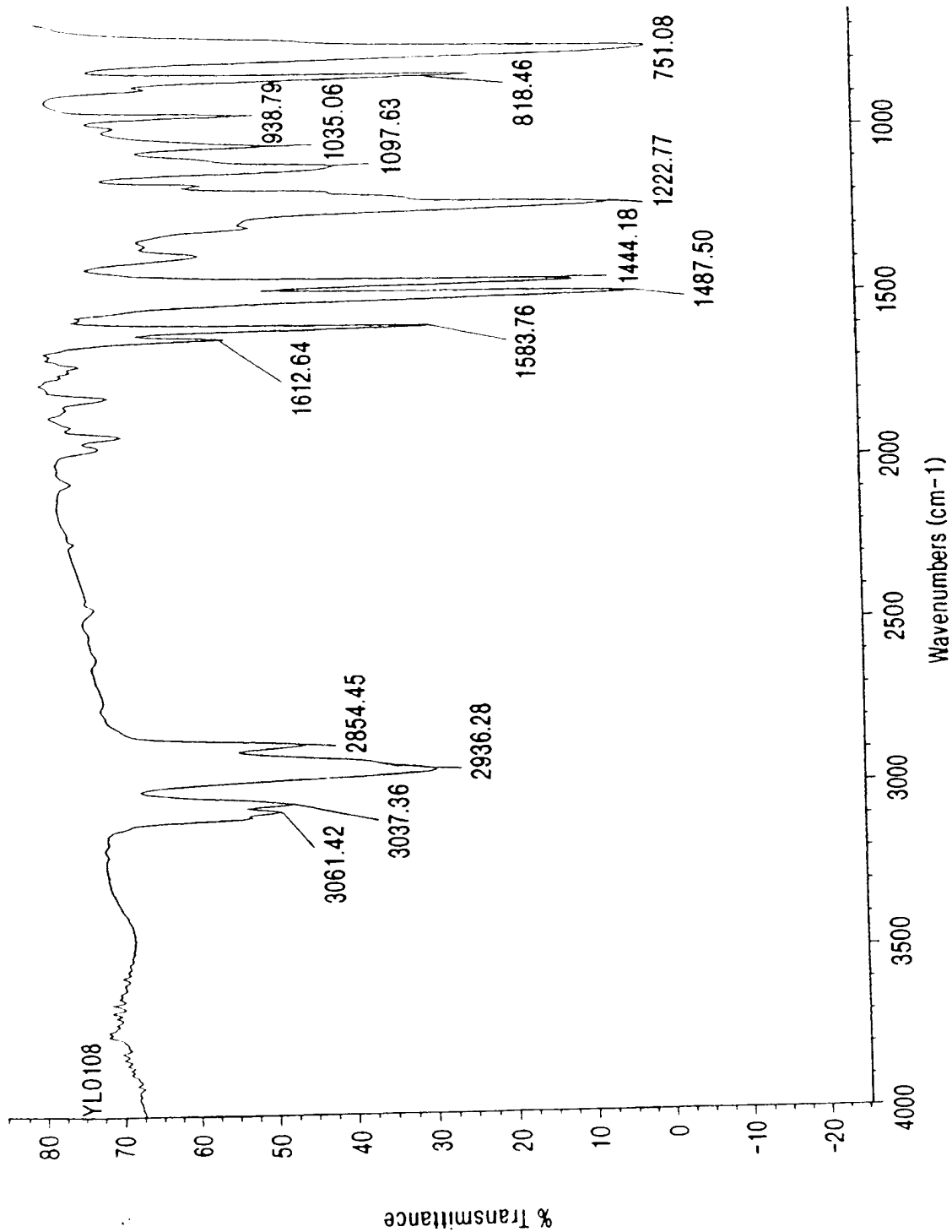
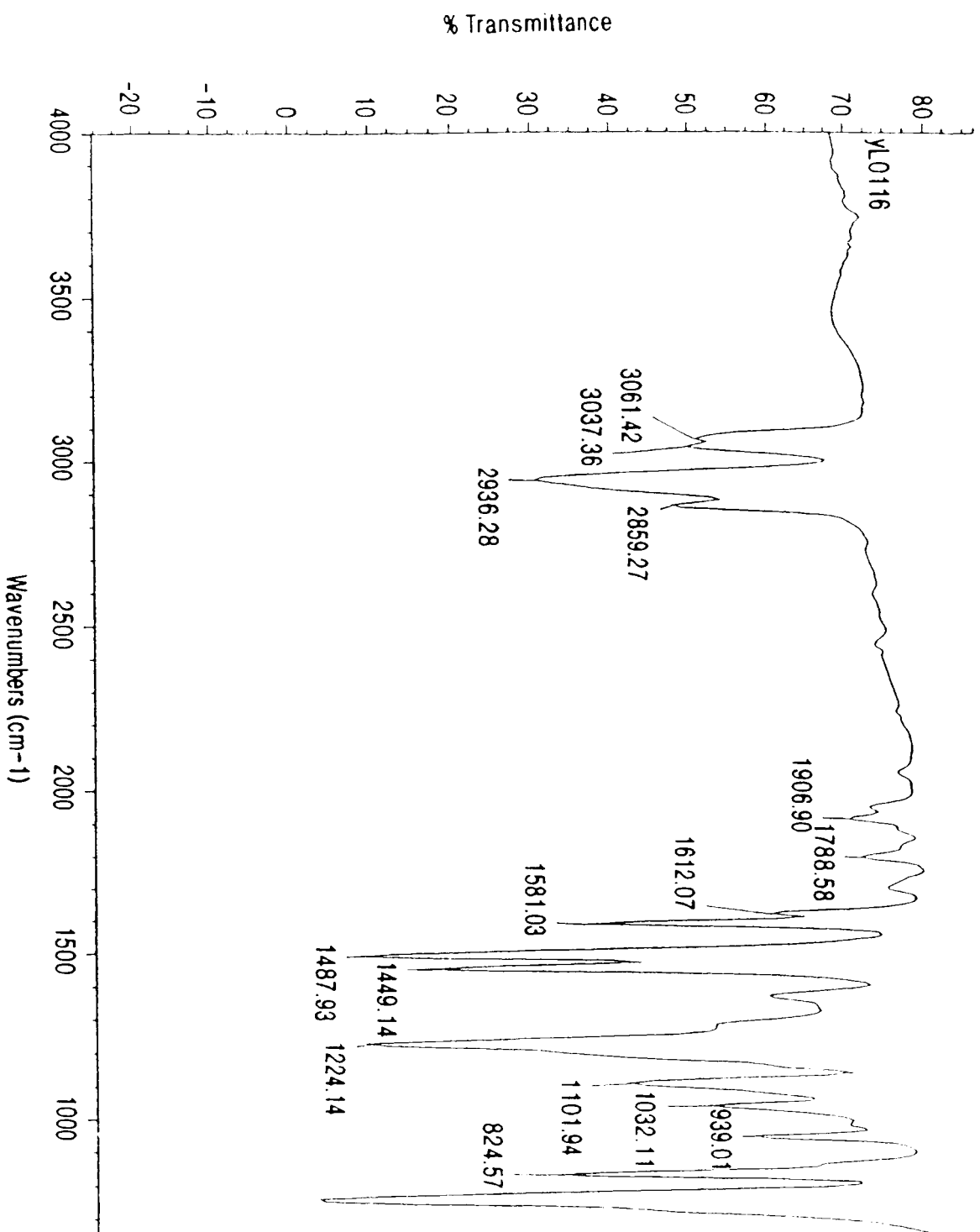


FIG. 95

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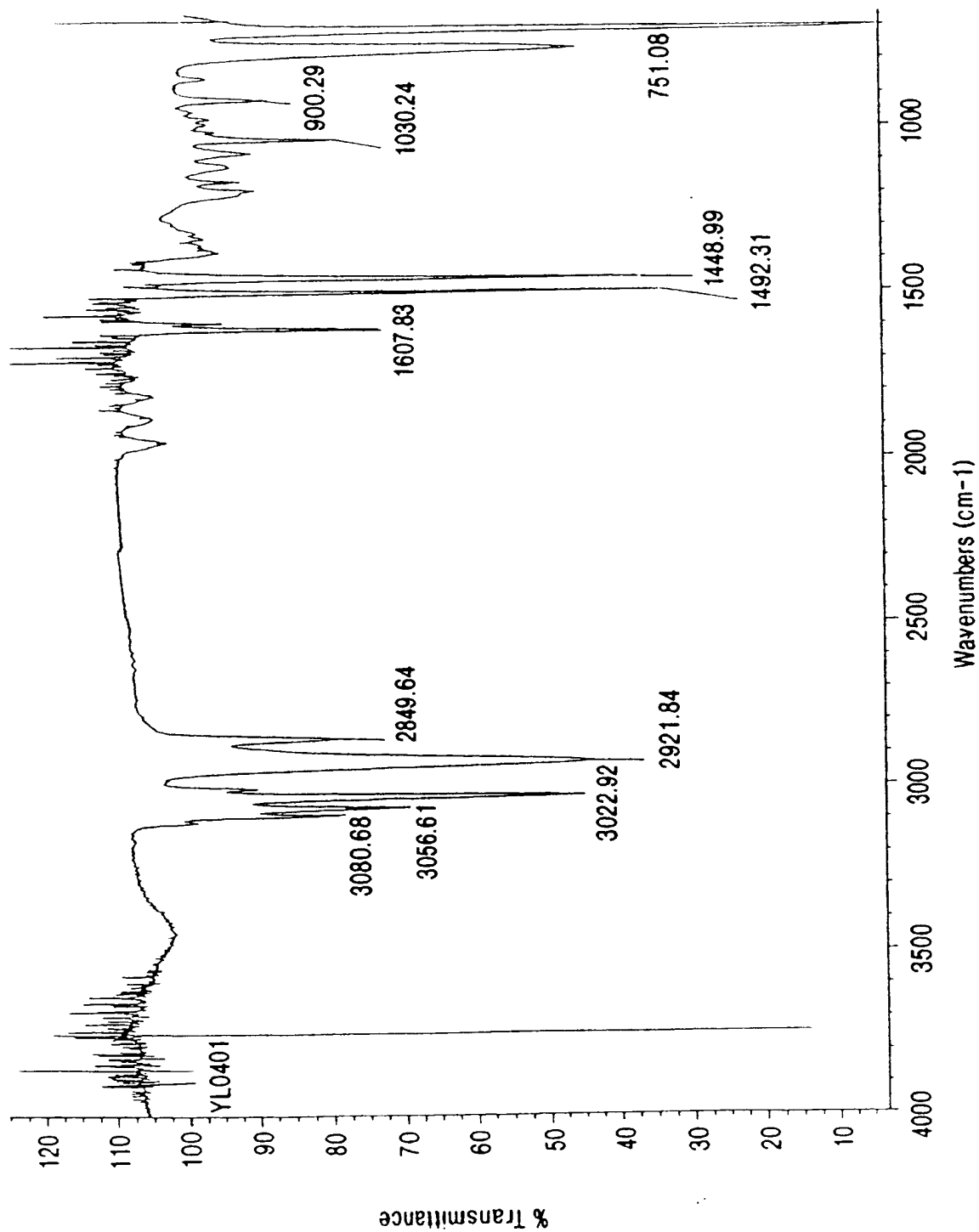
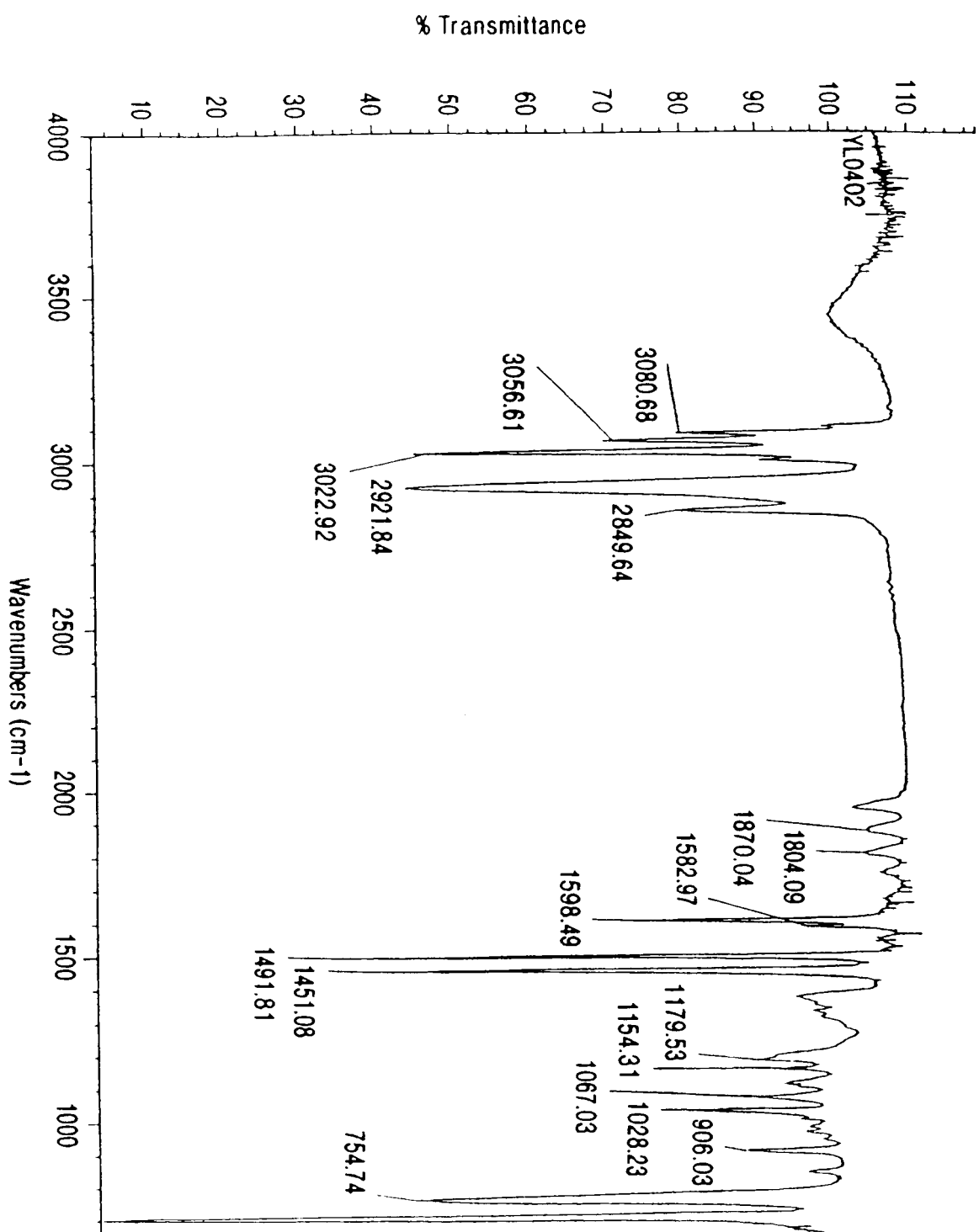


FIG. 97

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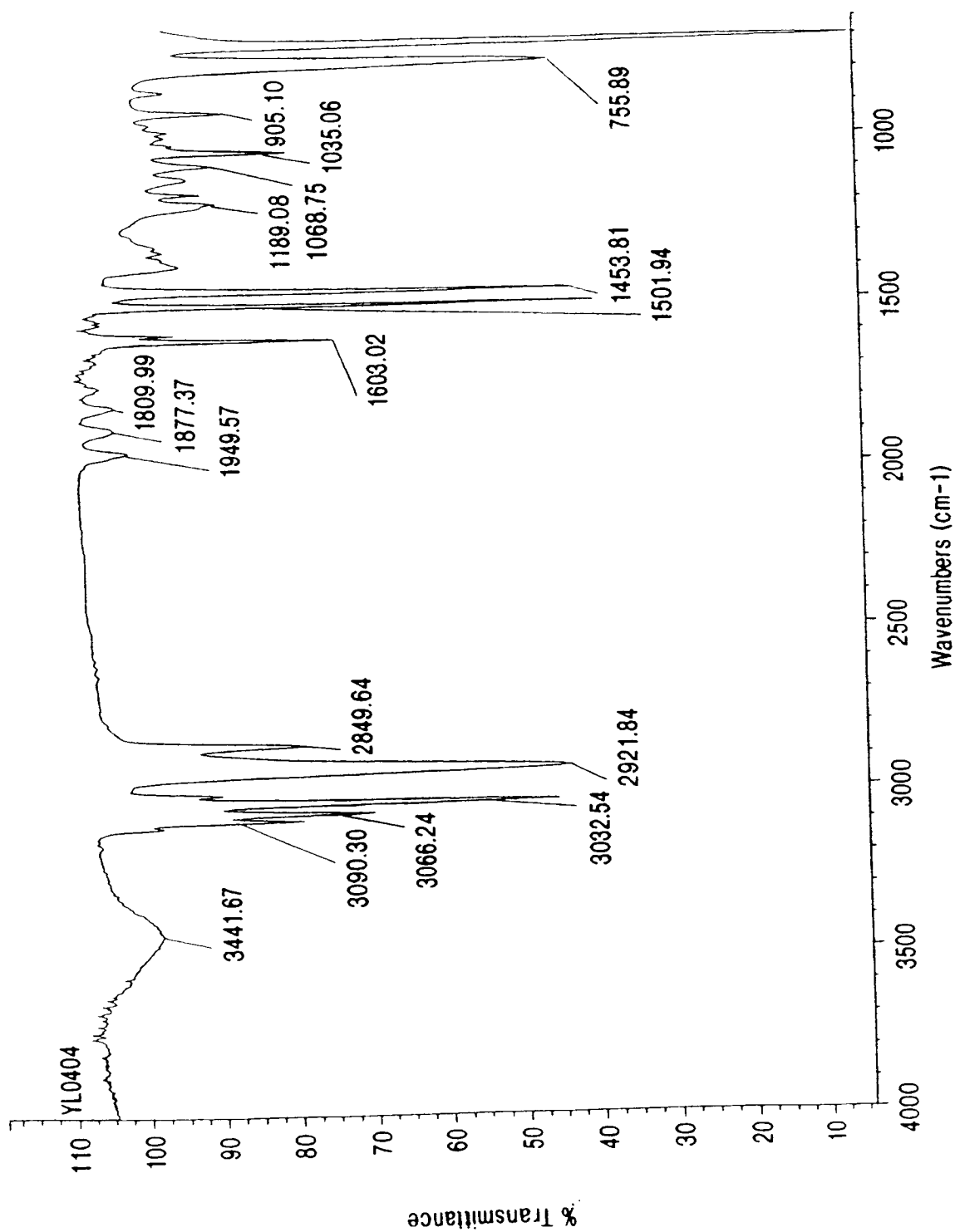


FIG. 99

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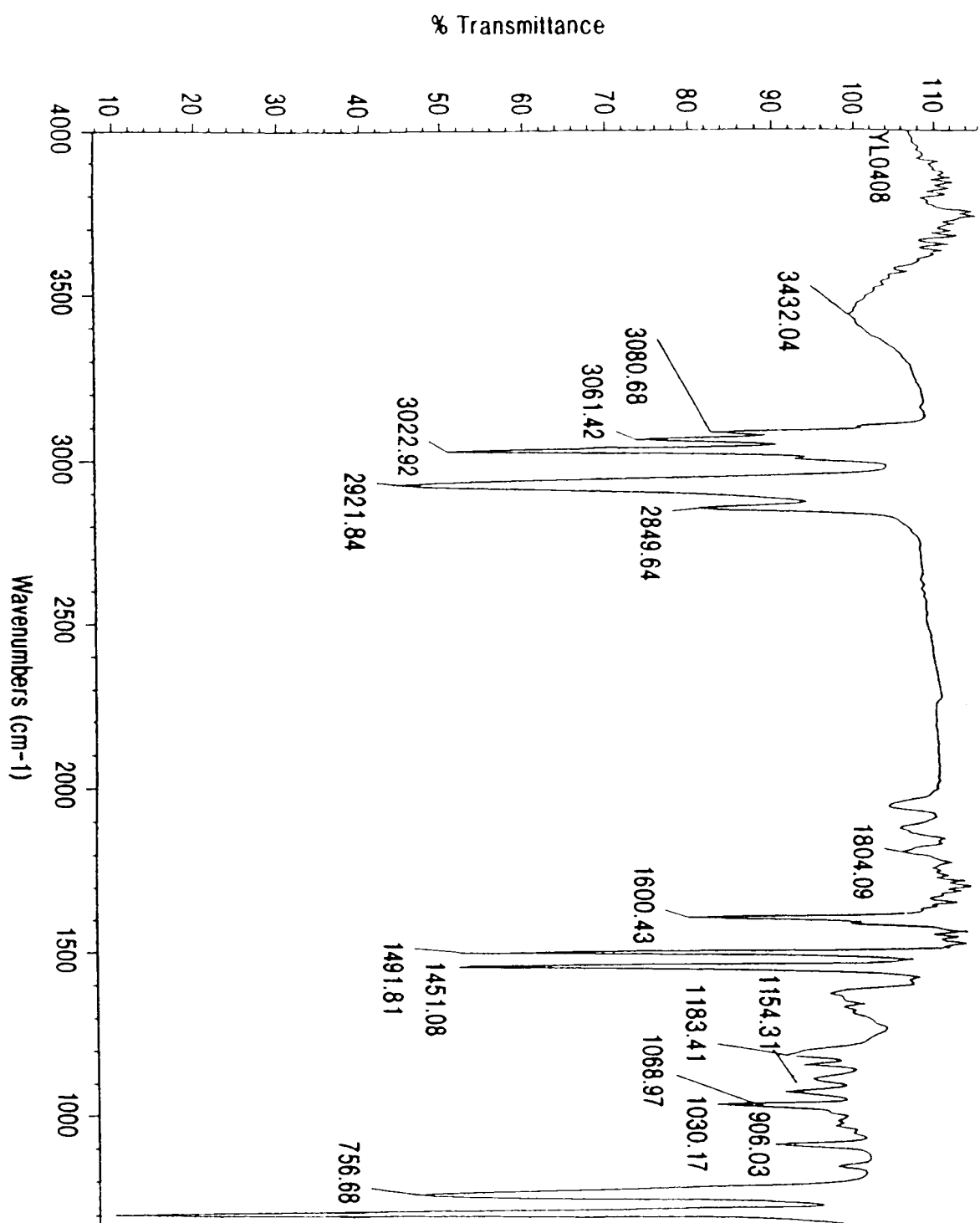


FIG. 100

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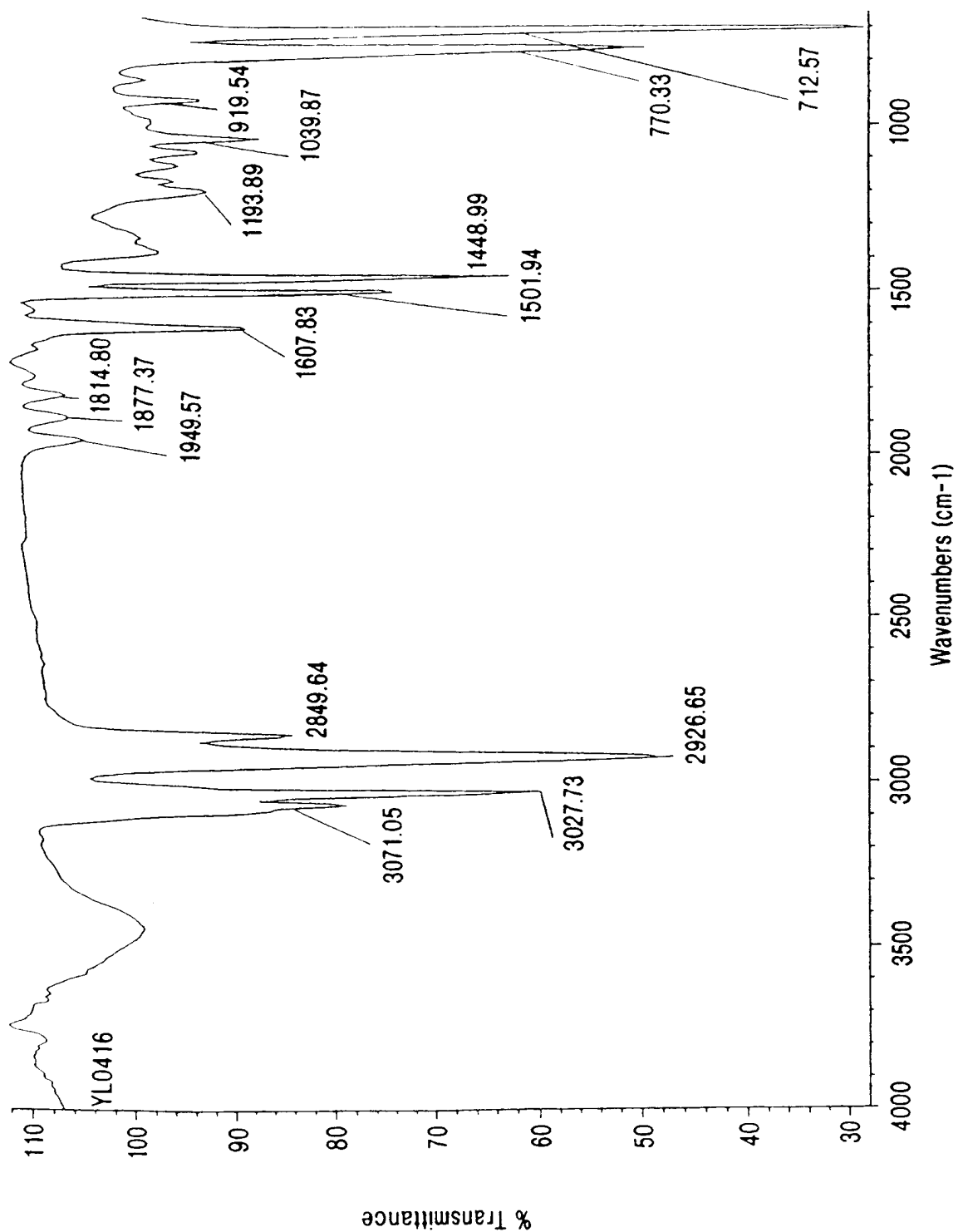


FIG. 101

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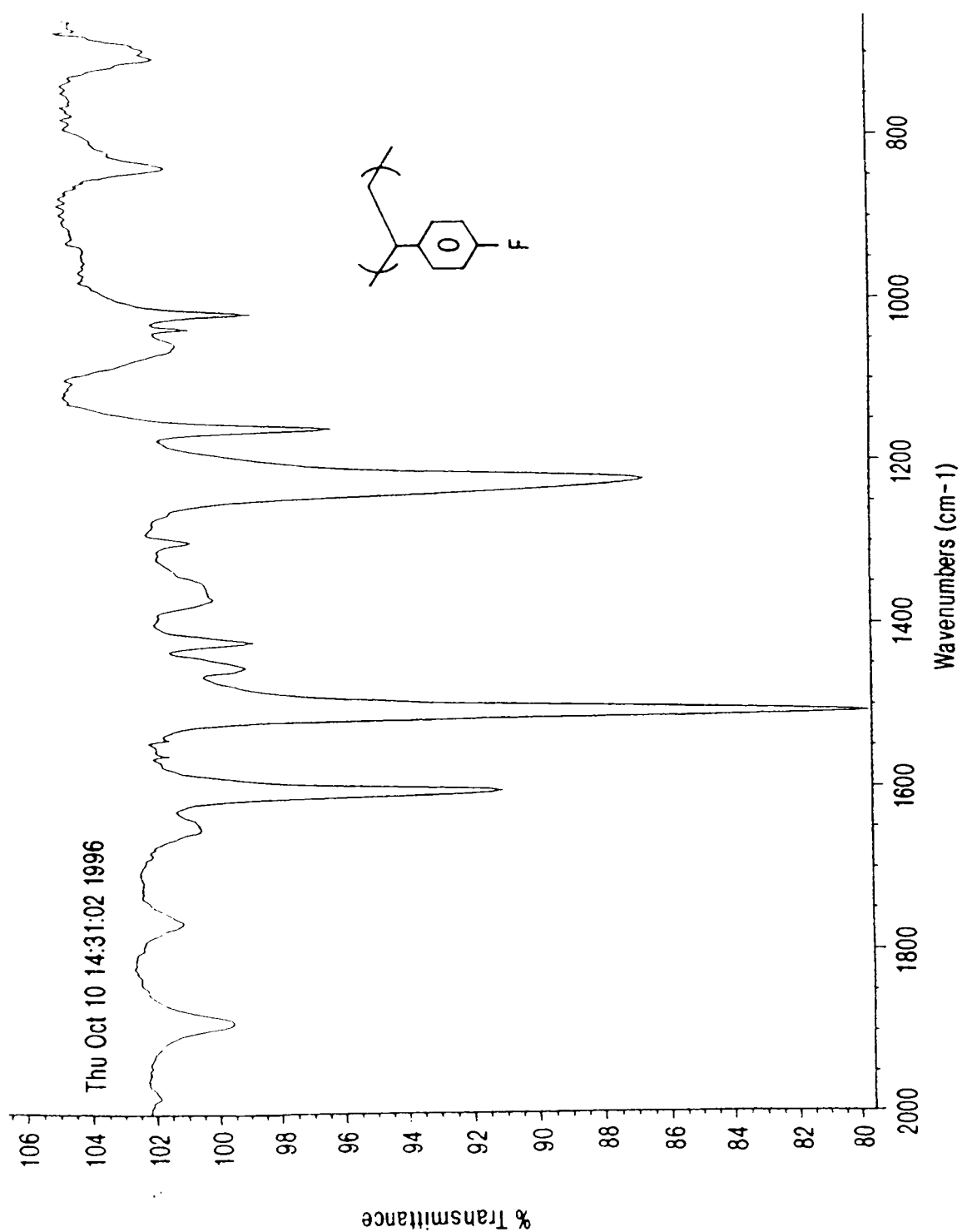


FIG. 102

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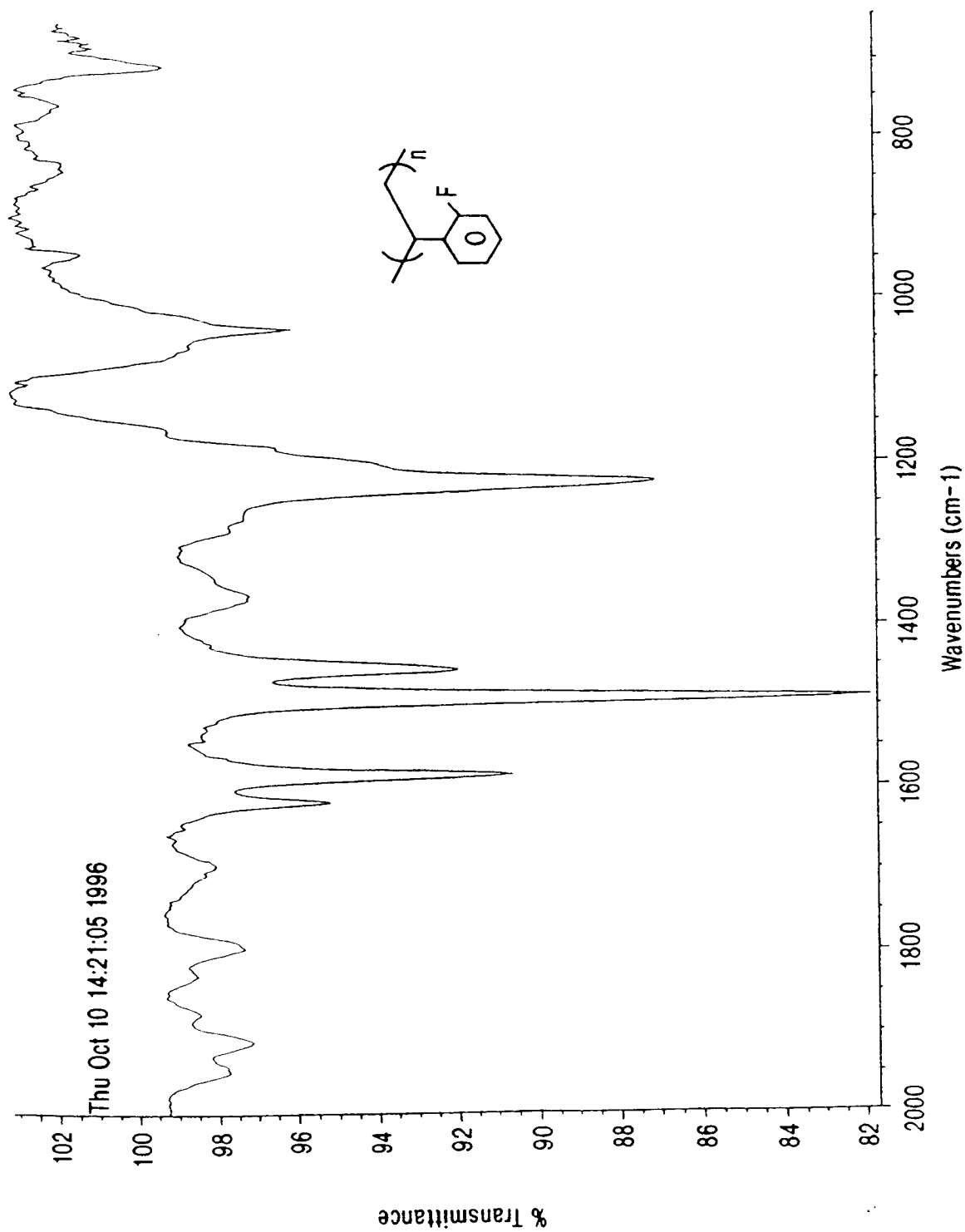


FIG. 103

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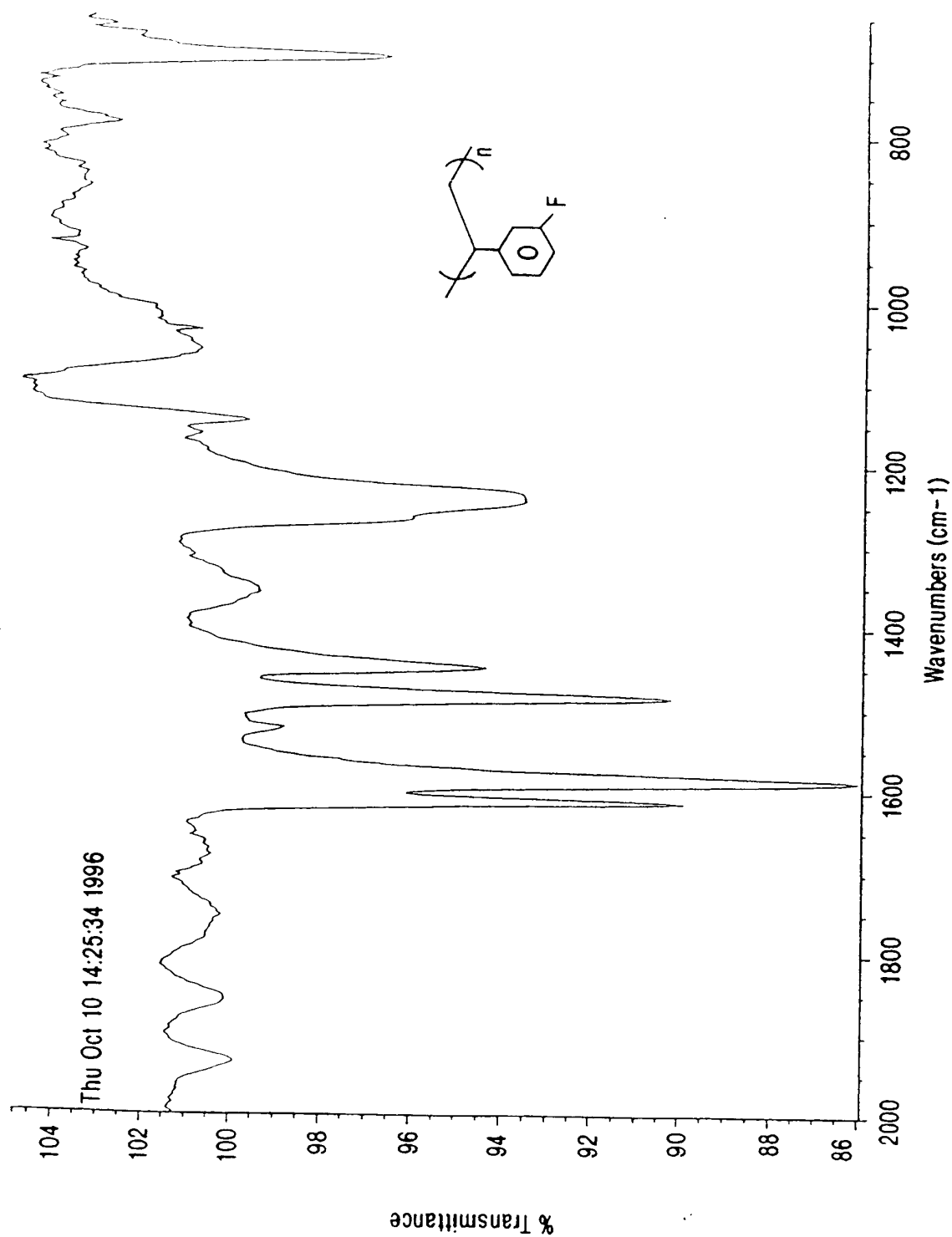


FIG. 104

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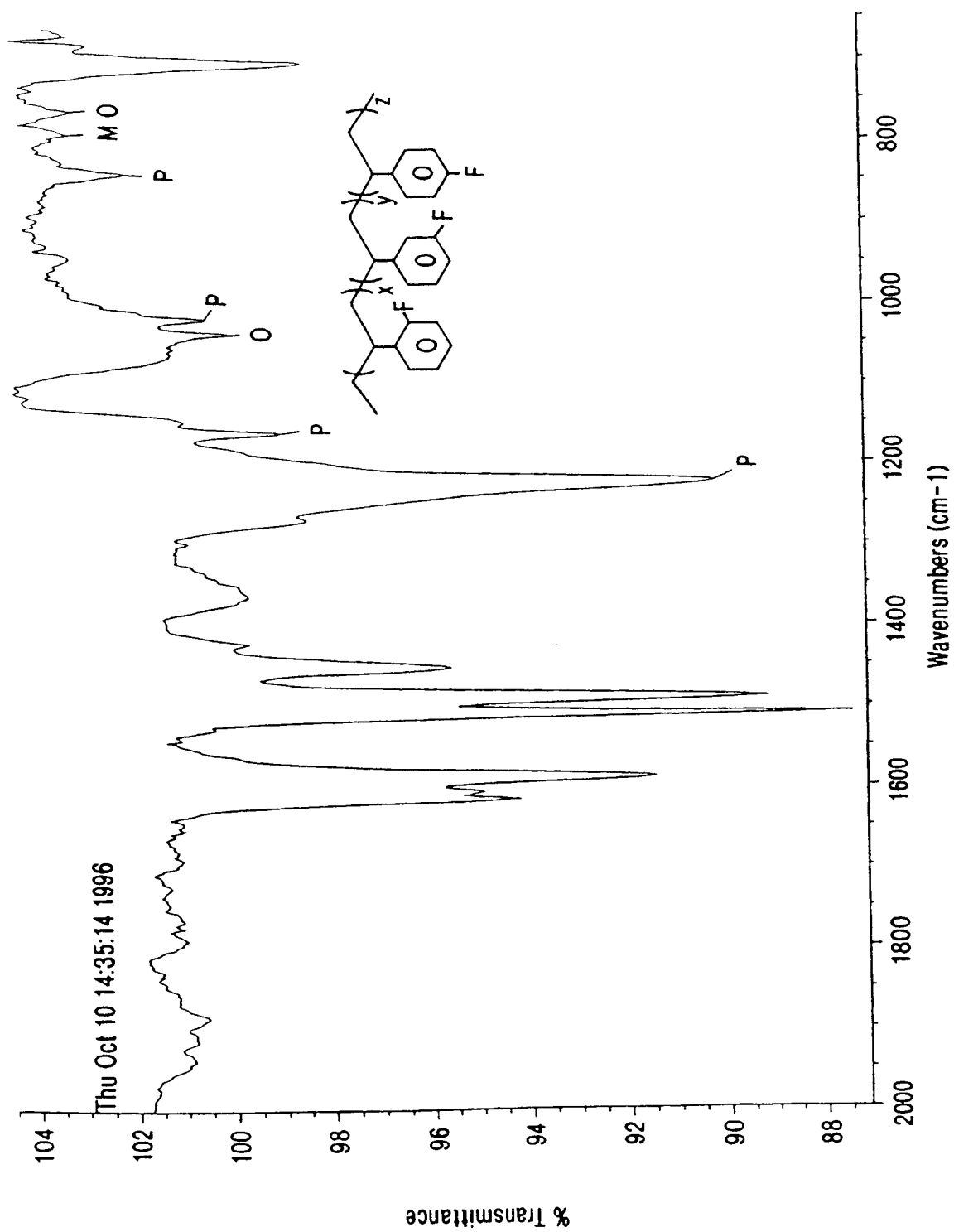


FIG. 105

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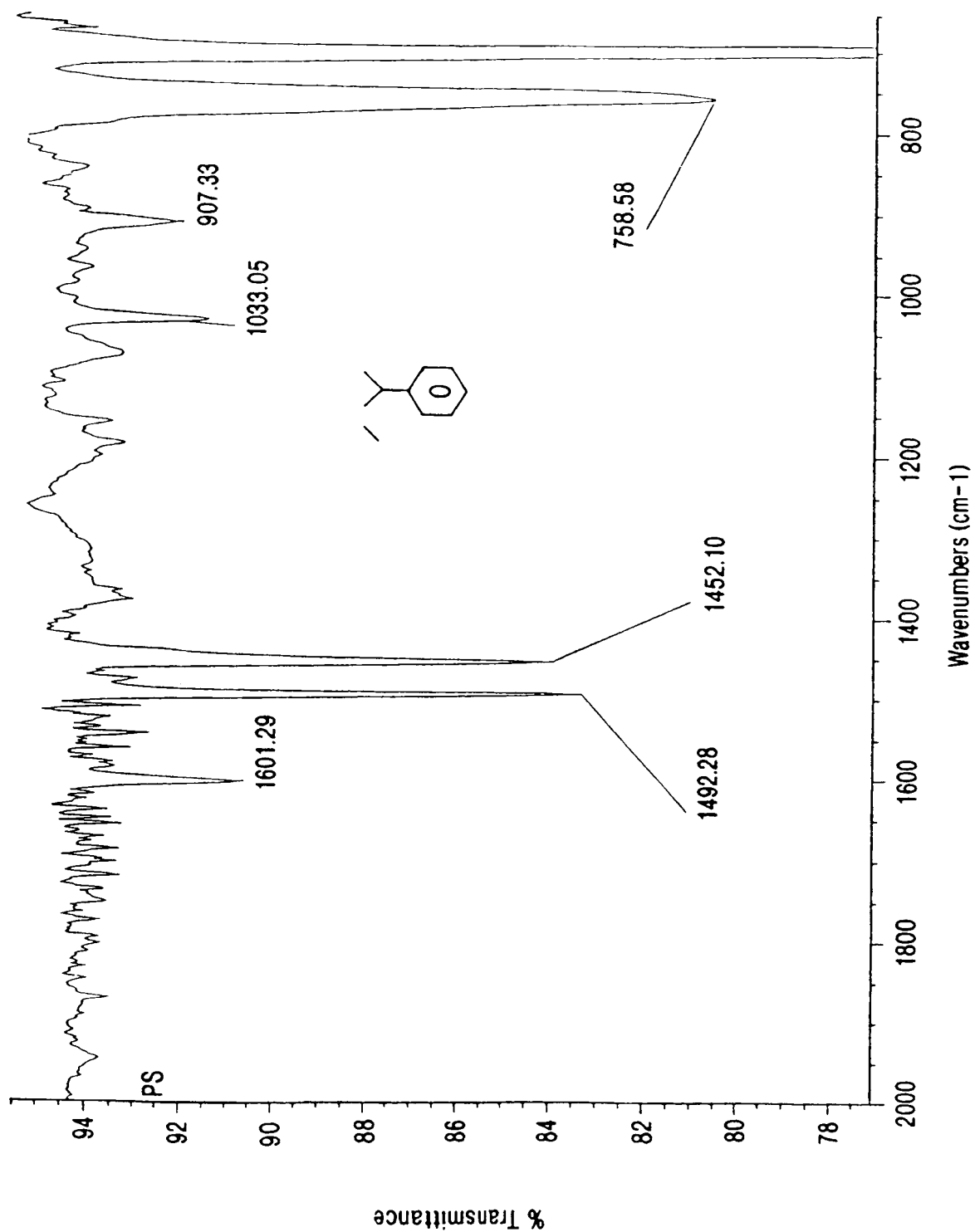
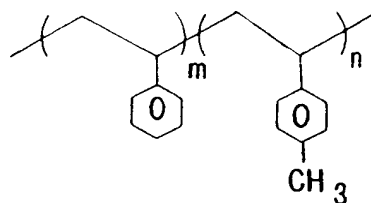
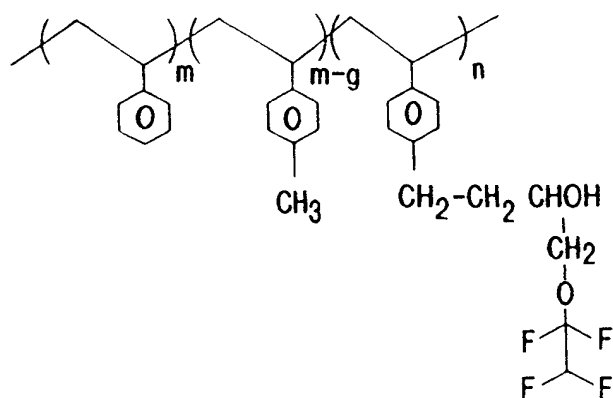
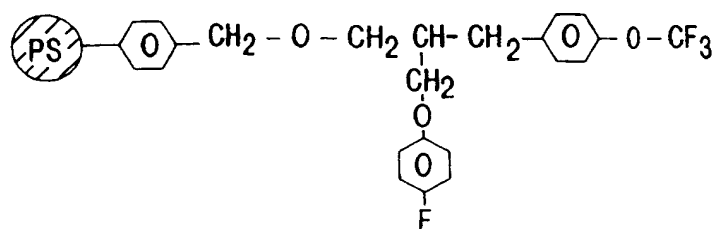
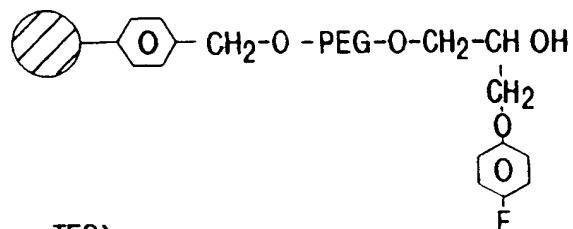


FIG. 106

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P (S-co-MS)

P(S-co-MS)-Rf₁PS-BOH-Rf₁-Rf₂PS-PEG-Rf₁

Poly(S-co-FS-co-TFS)

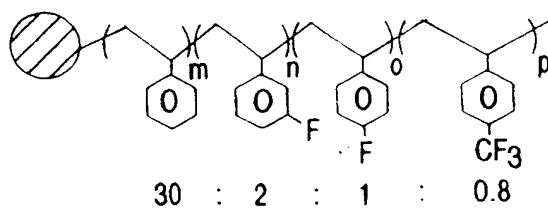


FIG. 107

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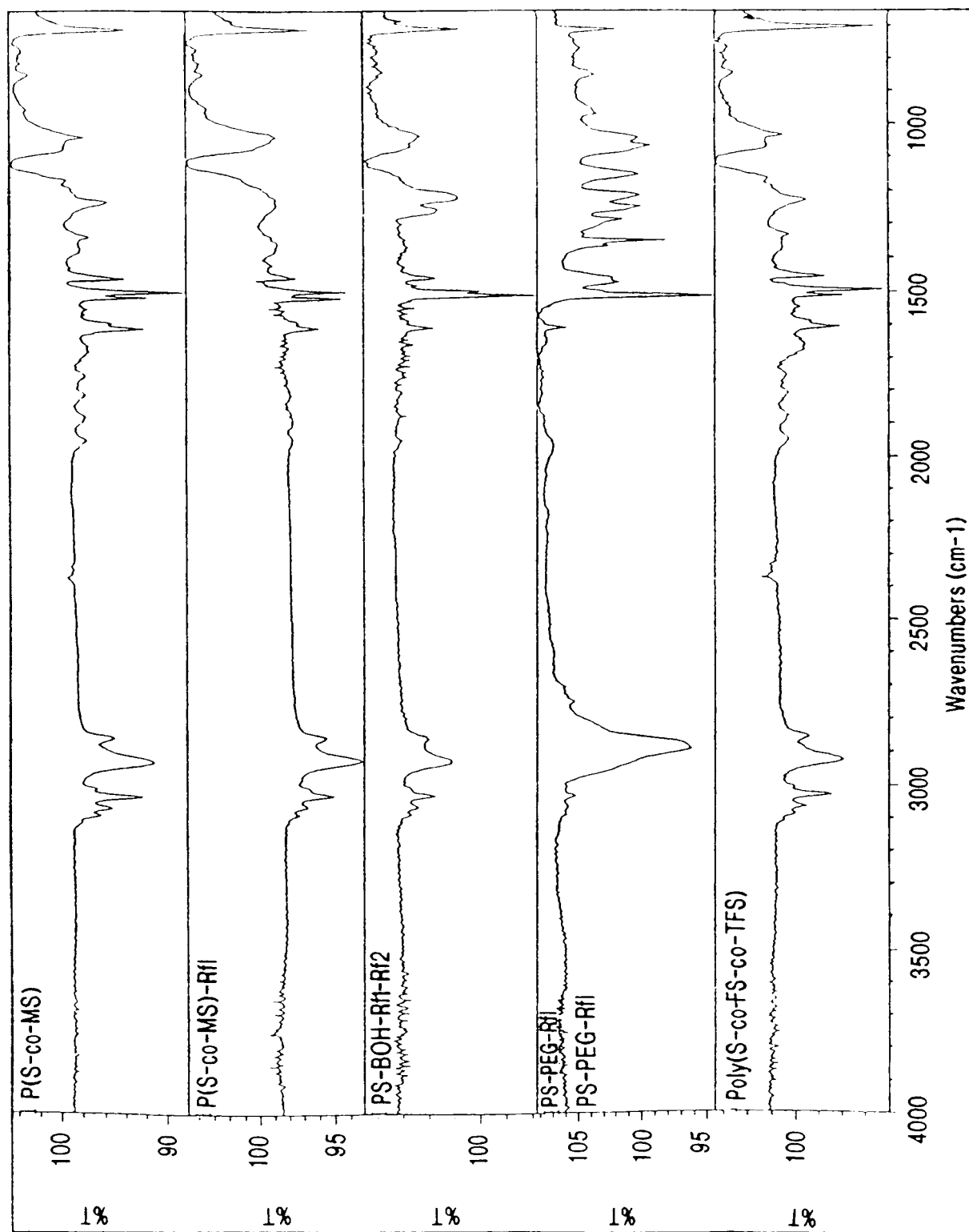


FIG. 108

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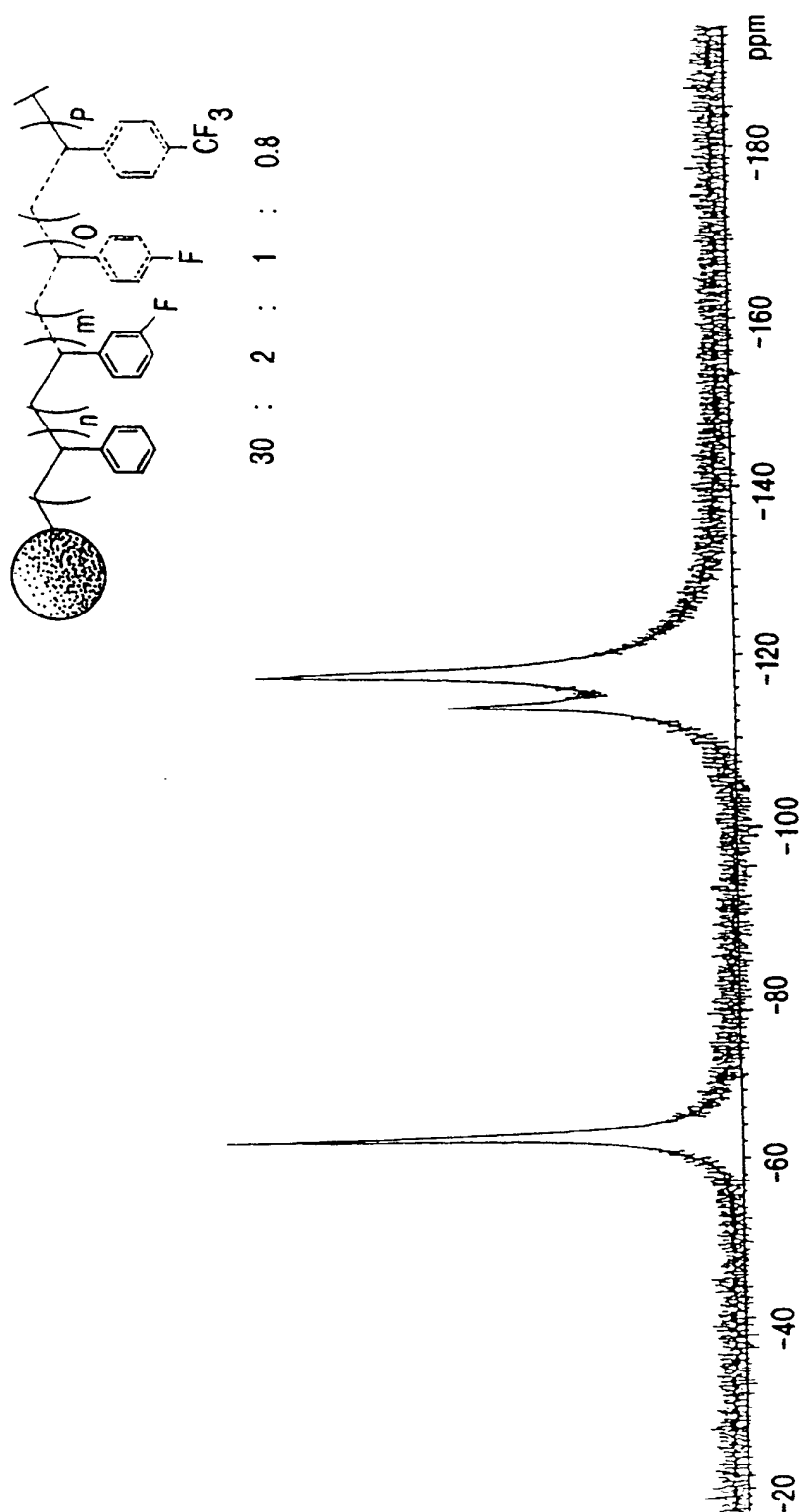


FIG. 109

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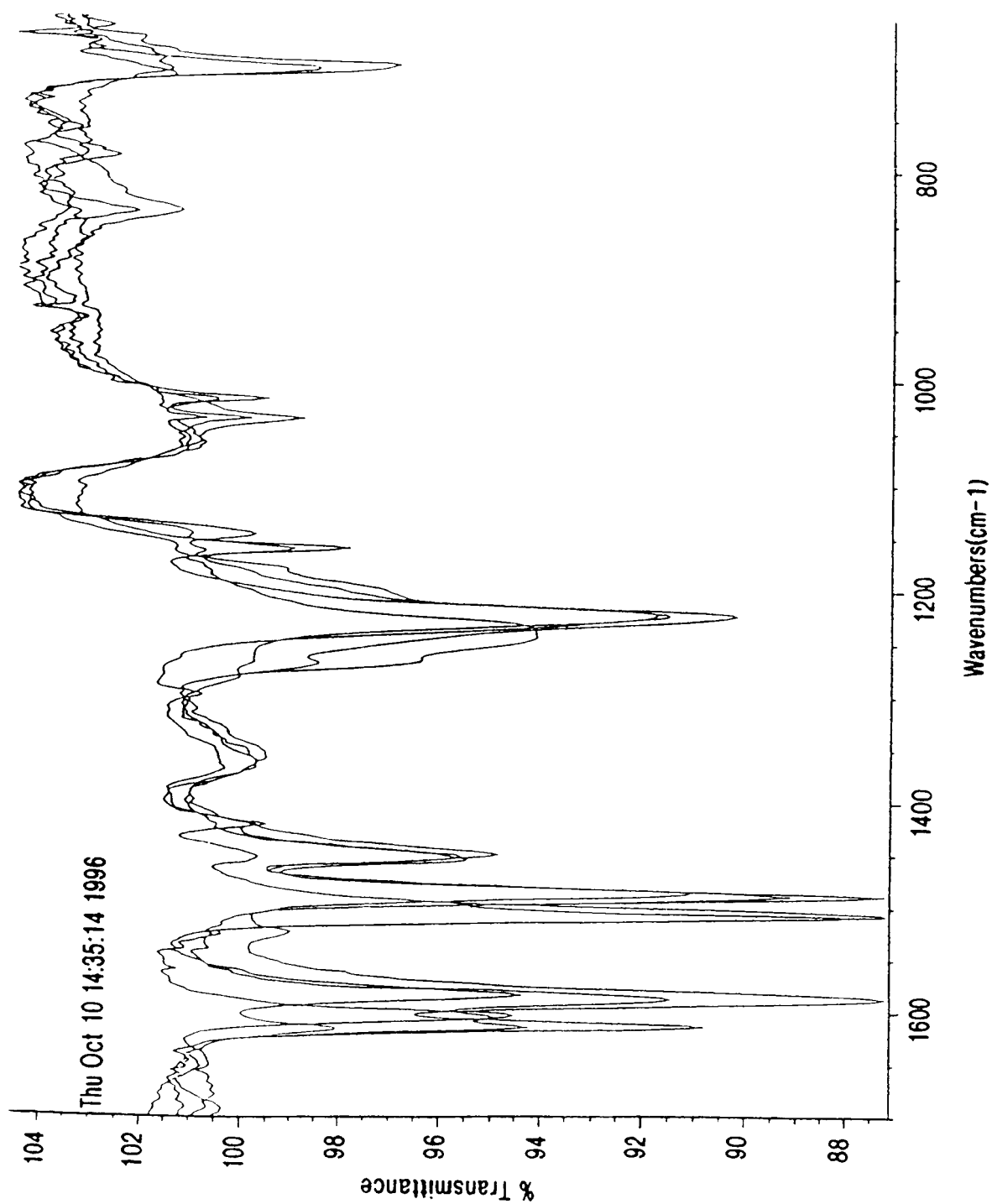


FIG. 110

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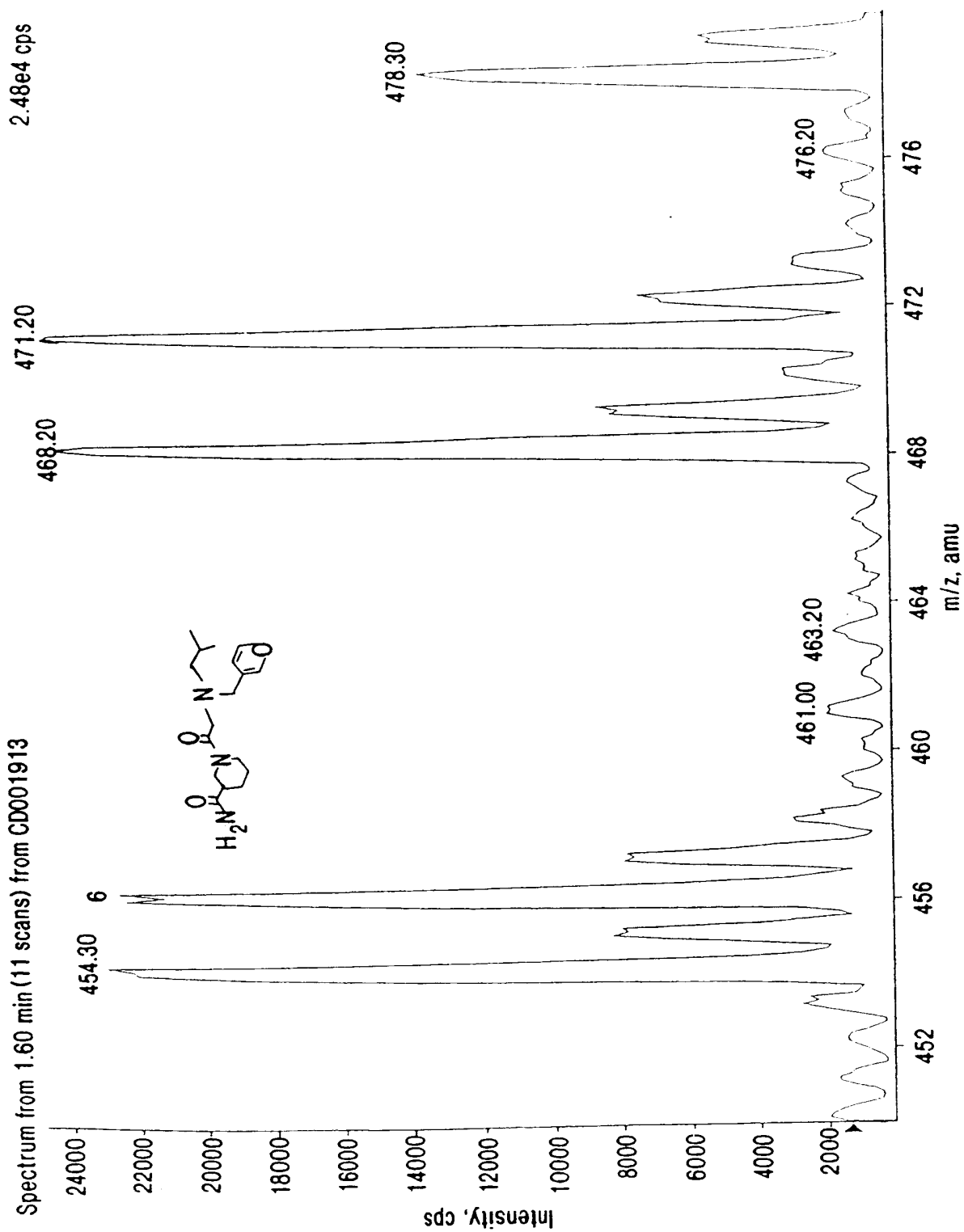


FIG. 111

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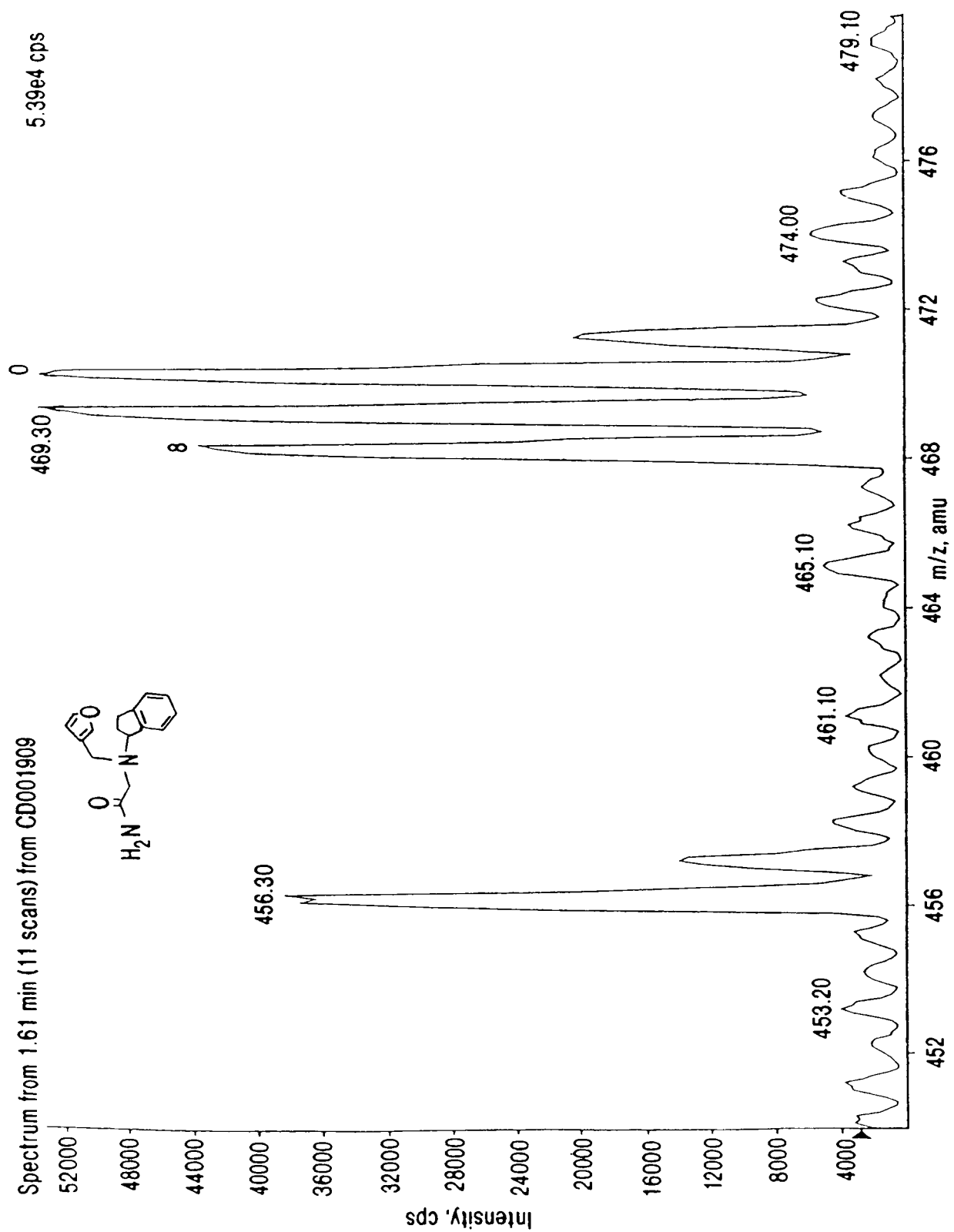


FIG. 112

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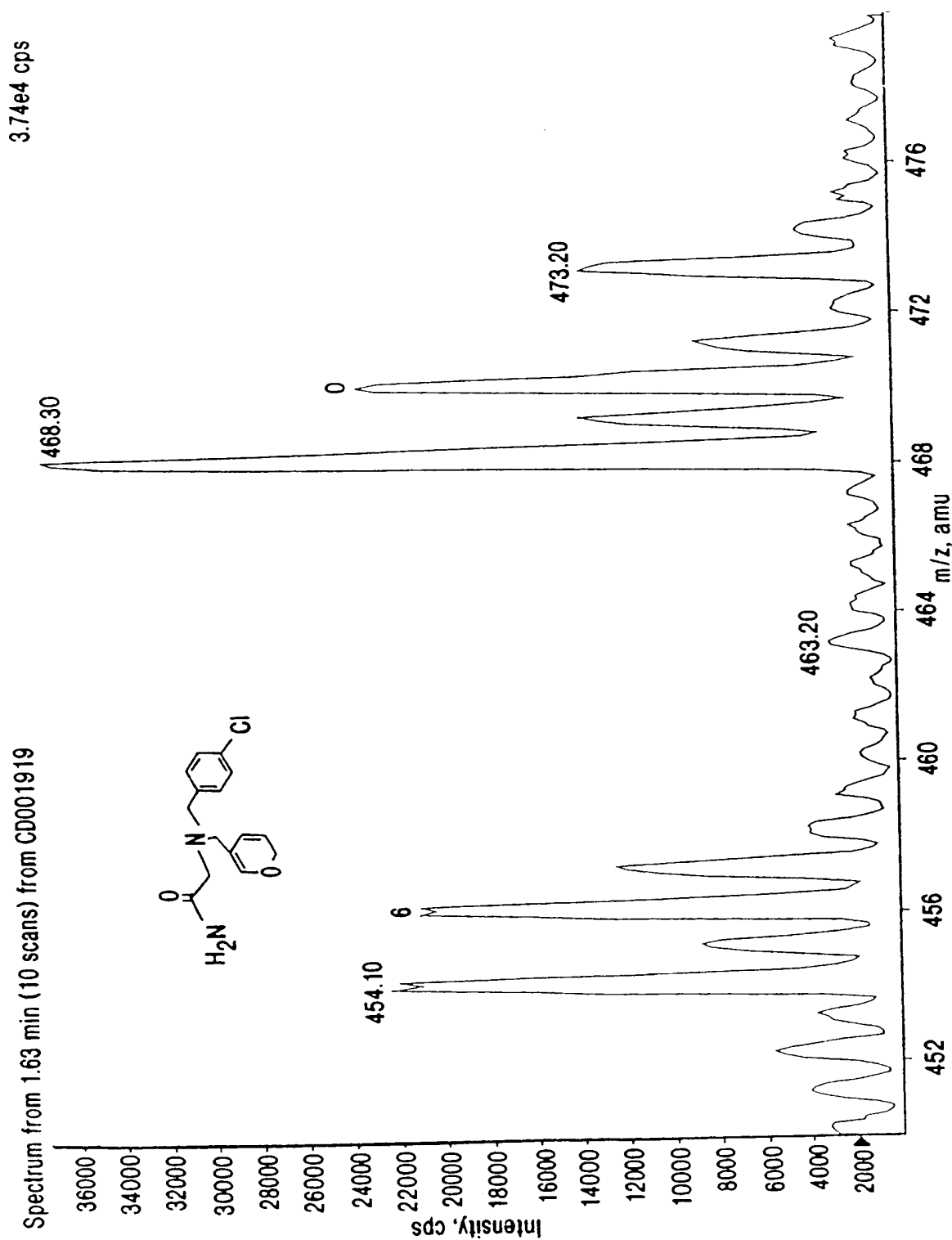


FIG. 113

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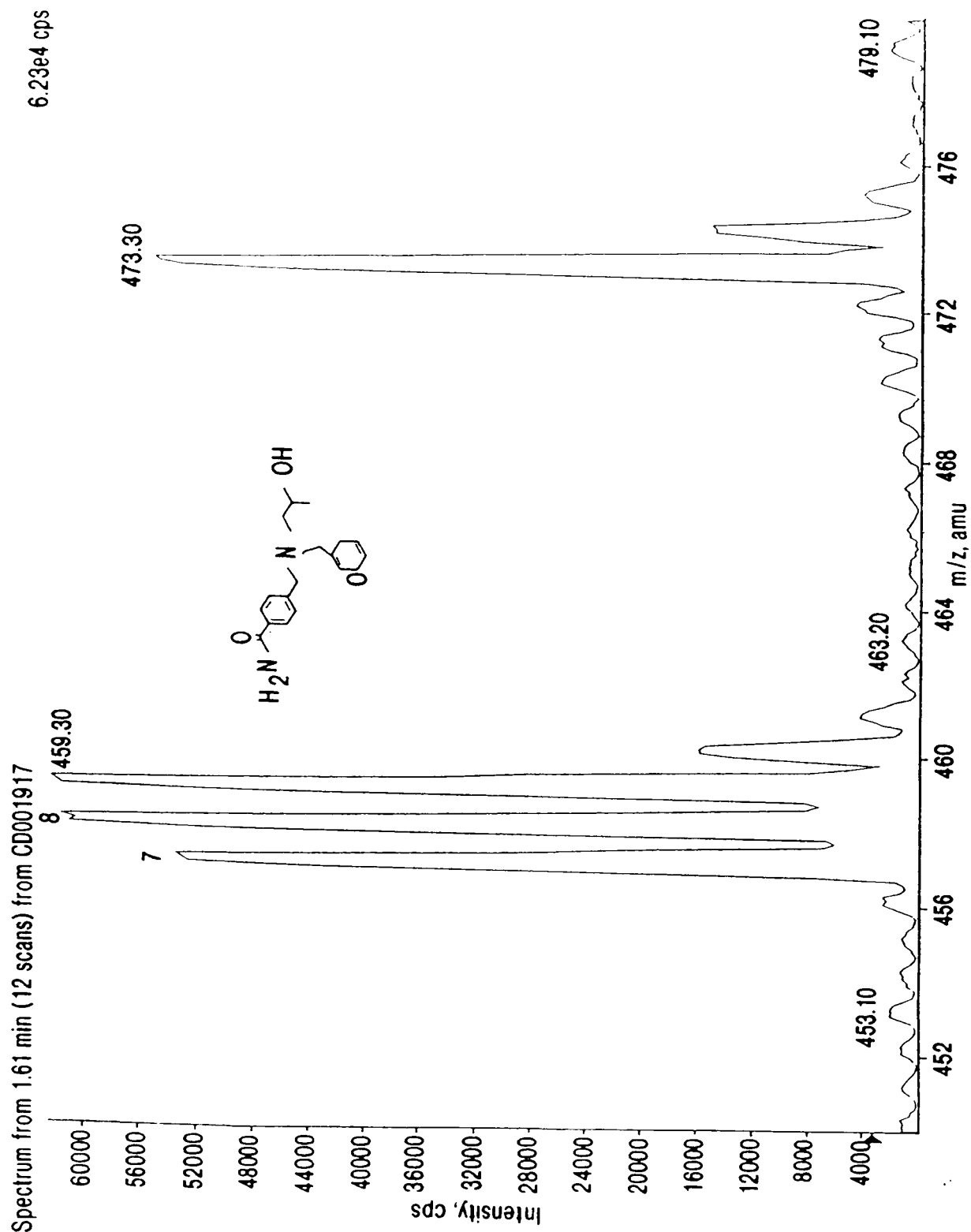


FIG. 114

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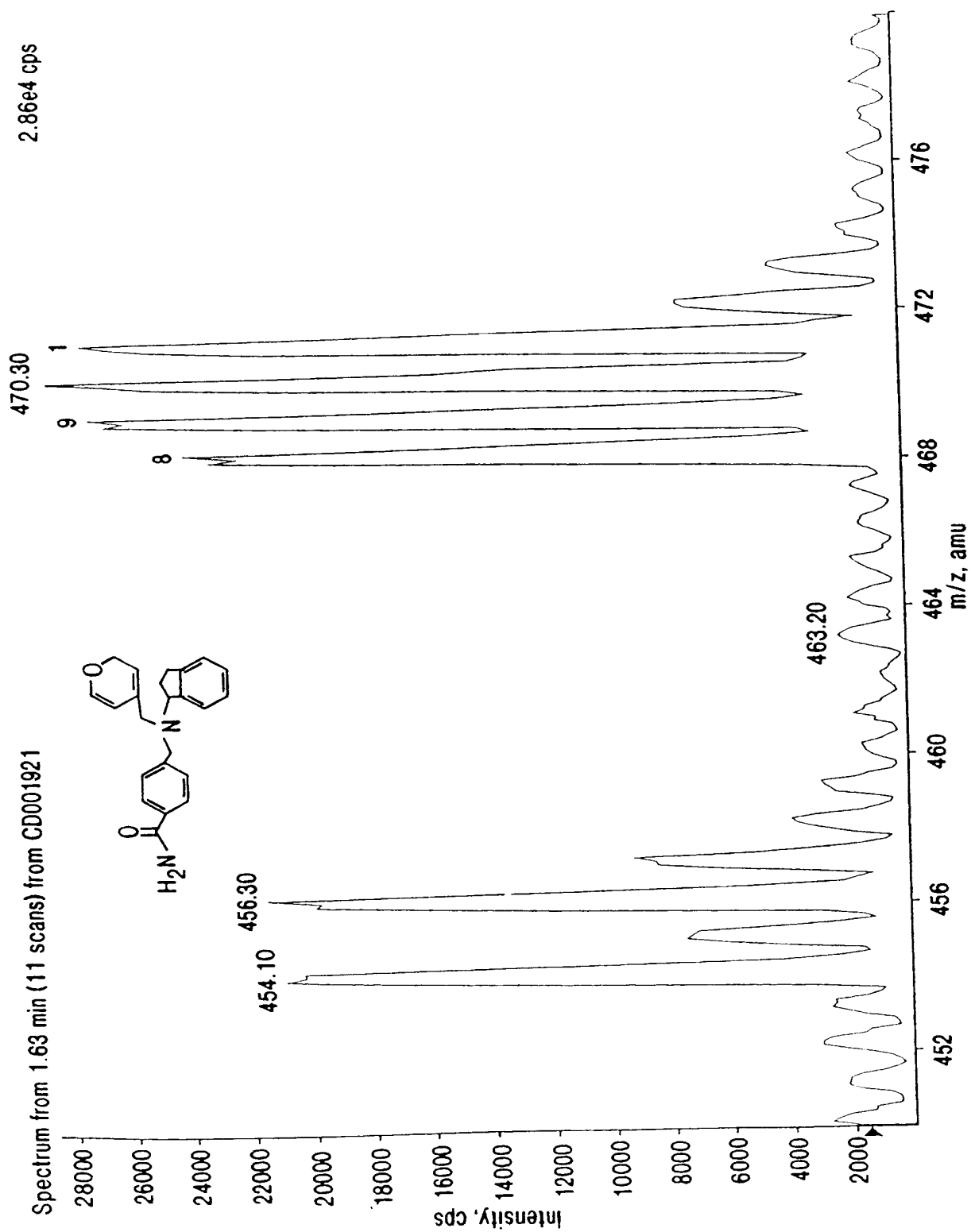


FIG. 115

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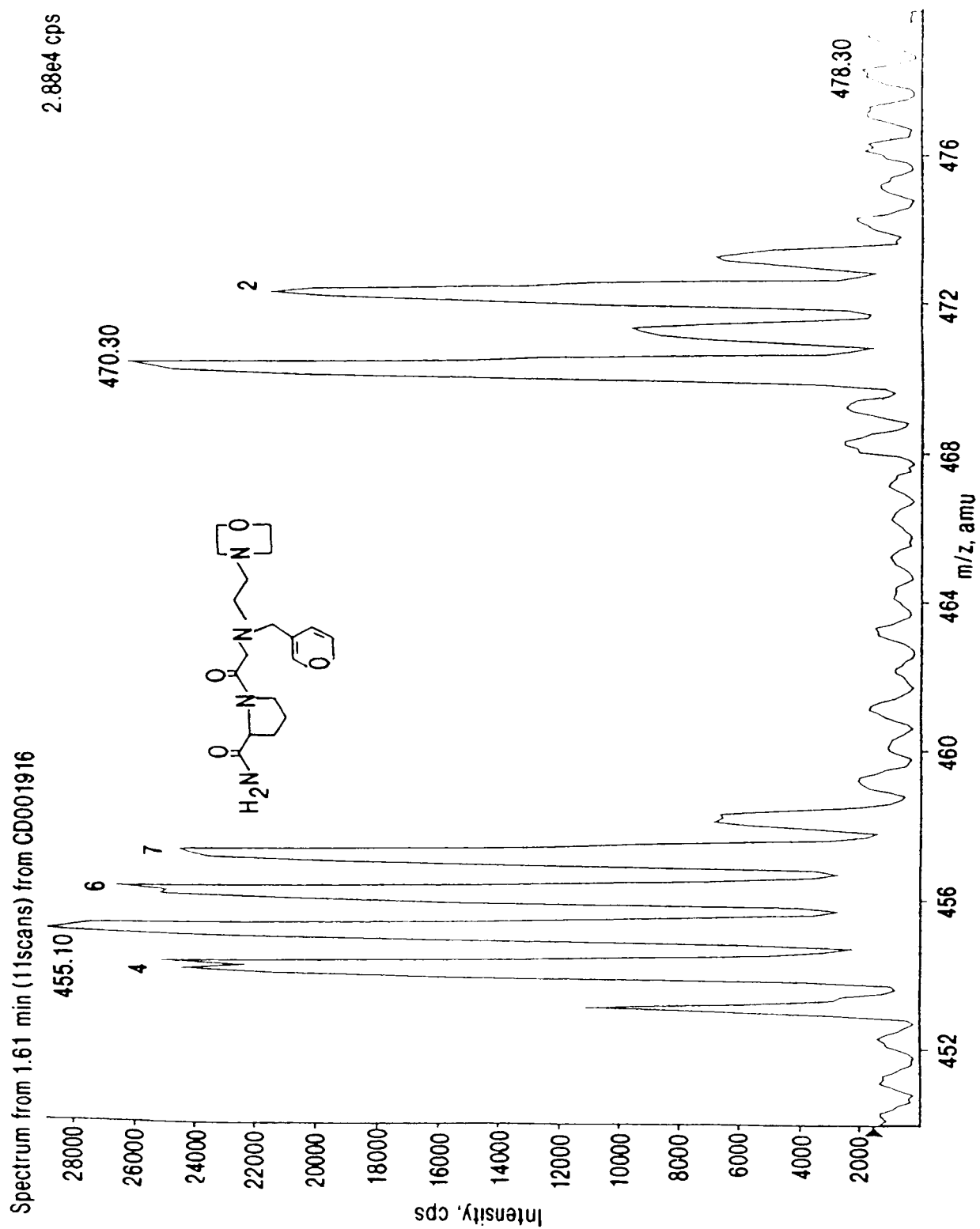


FIG. 116

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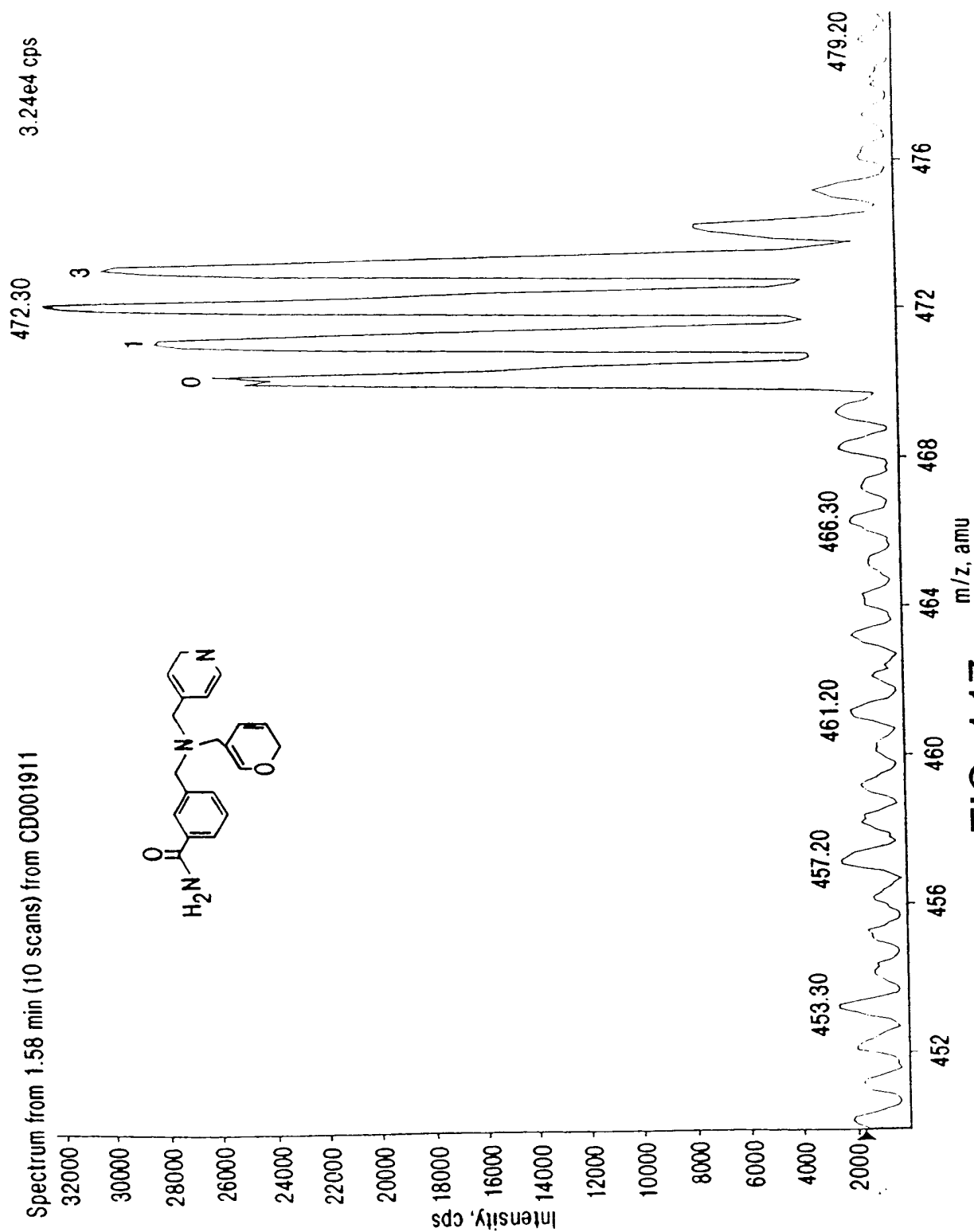


FIG. 117

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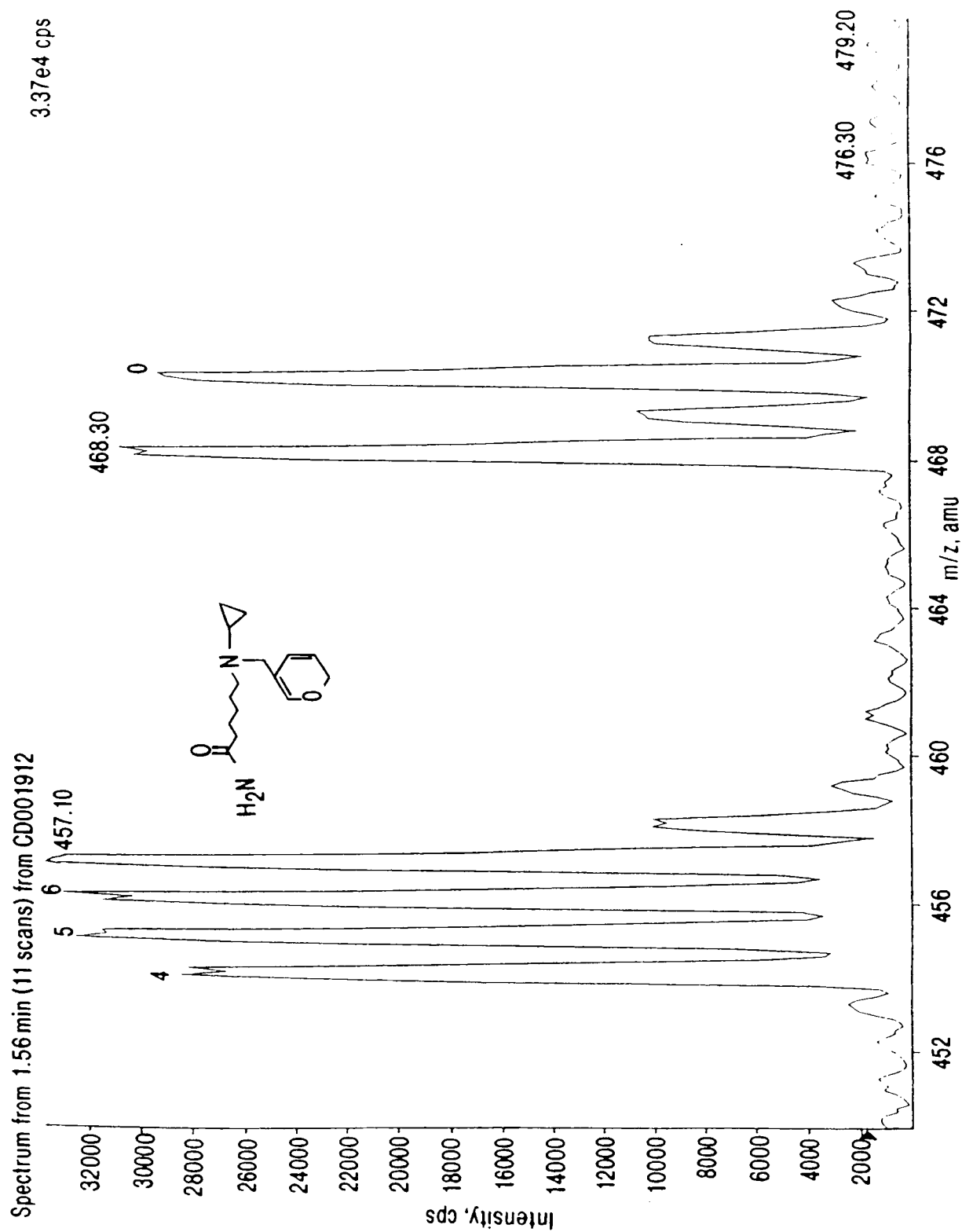


FIG. 118

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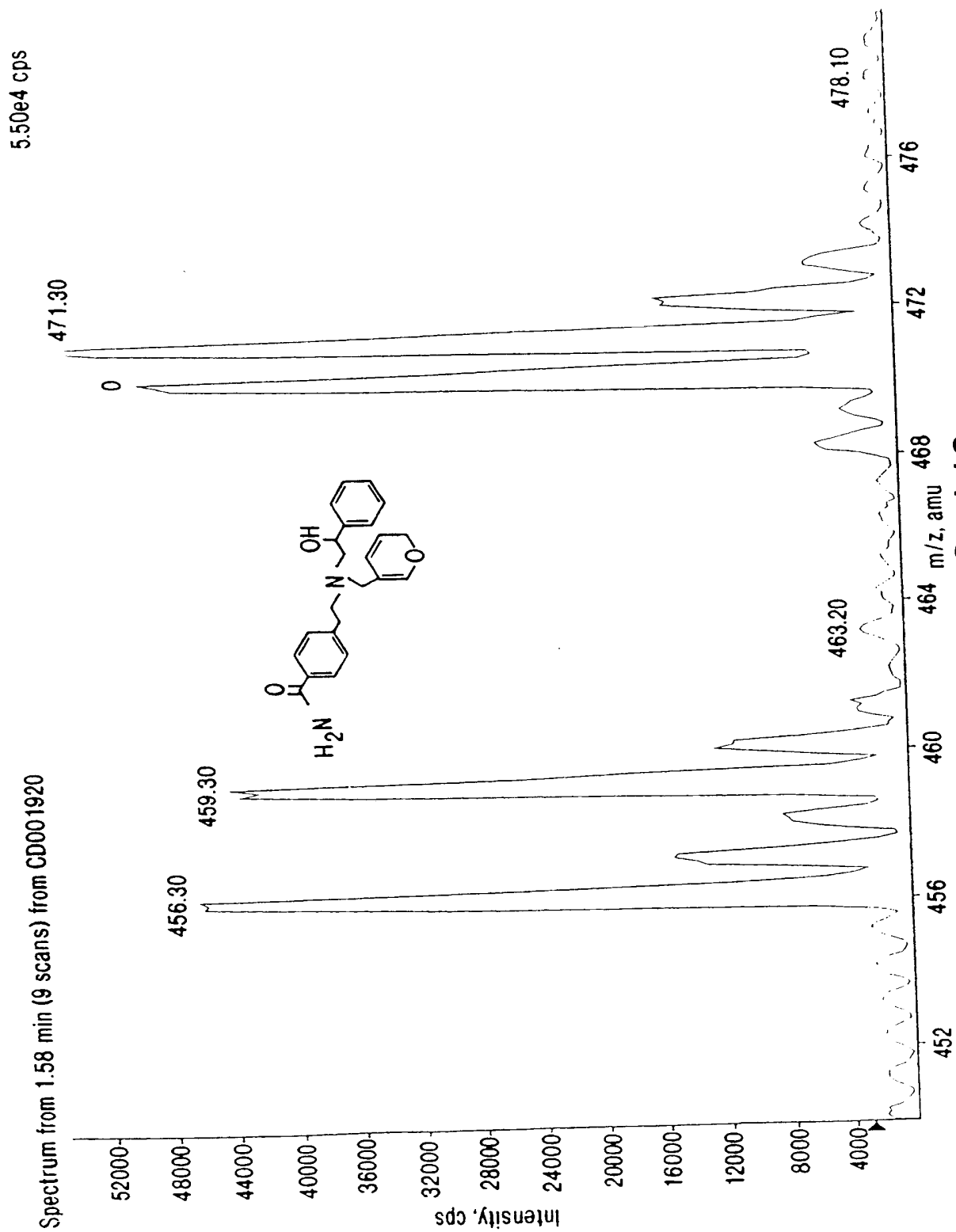


FIG. 119

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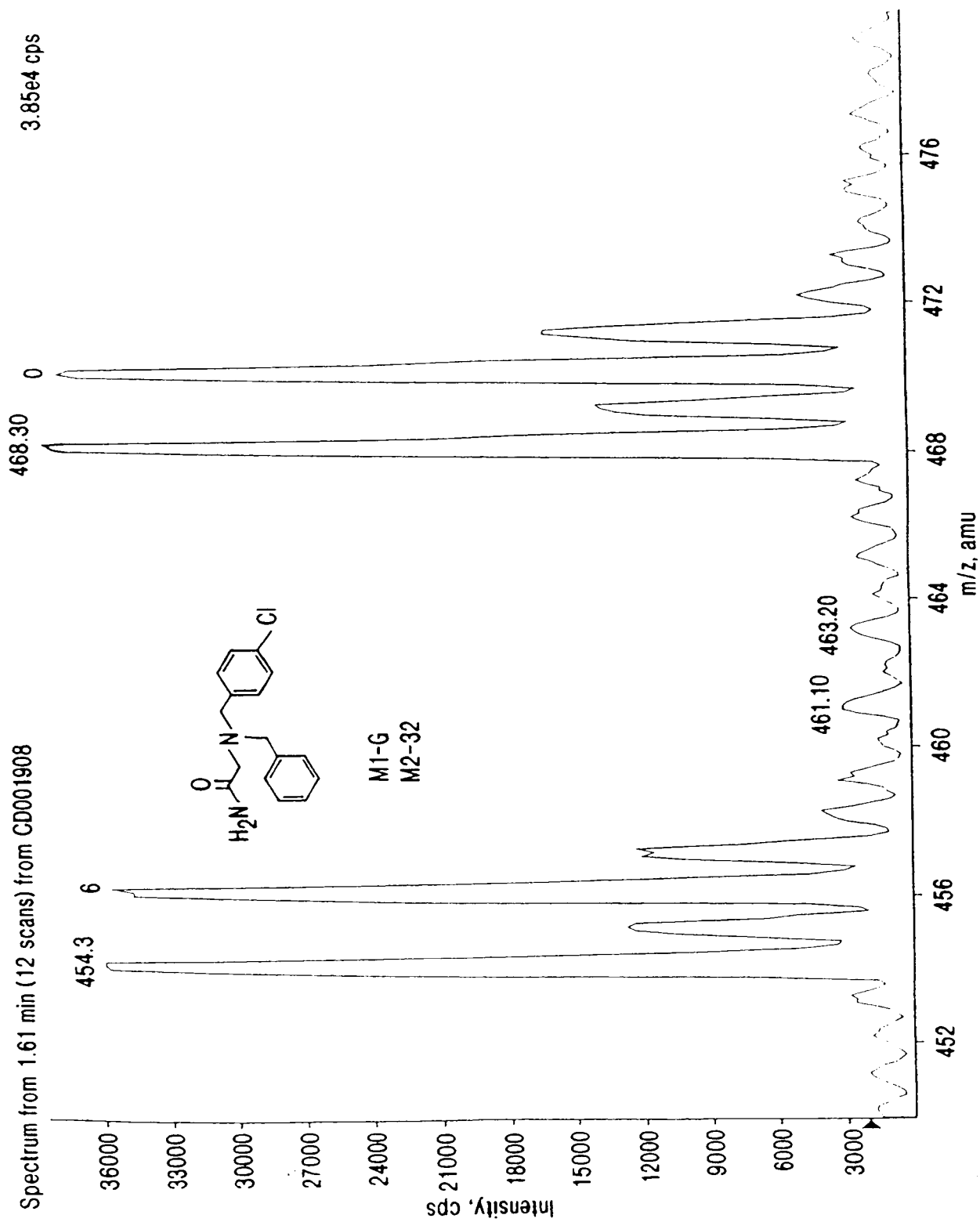


FIG. 120

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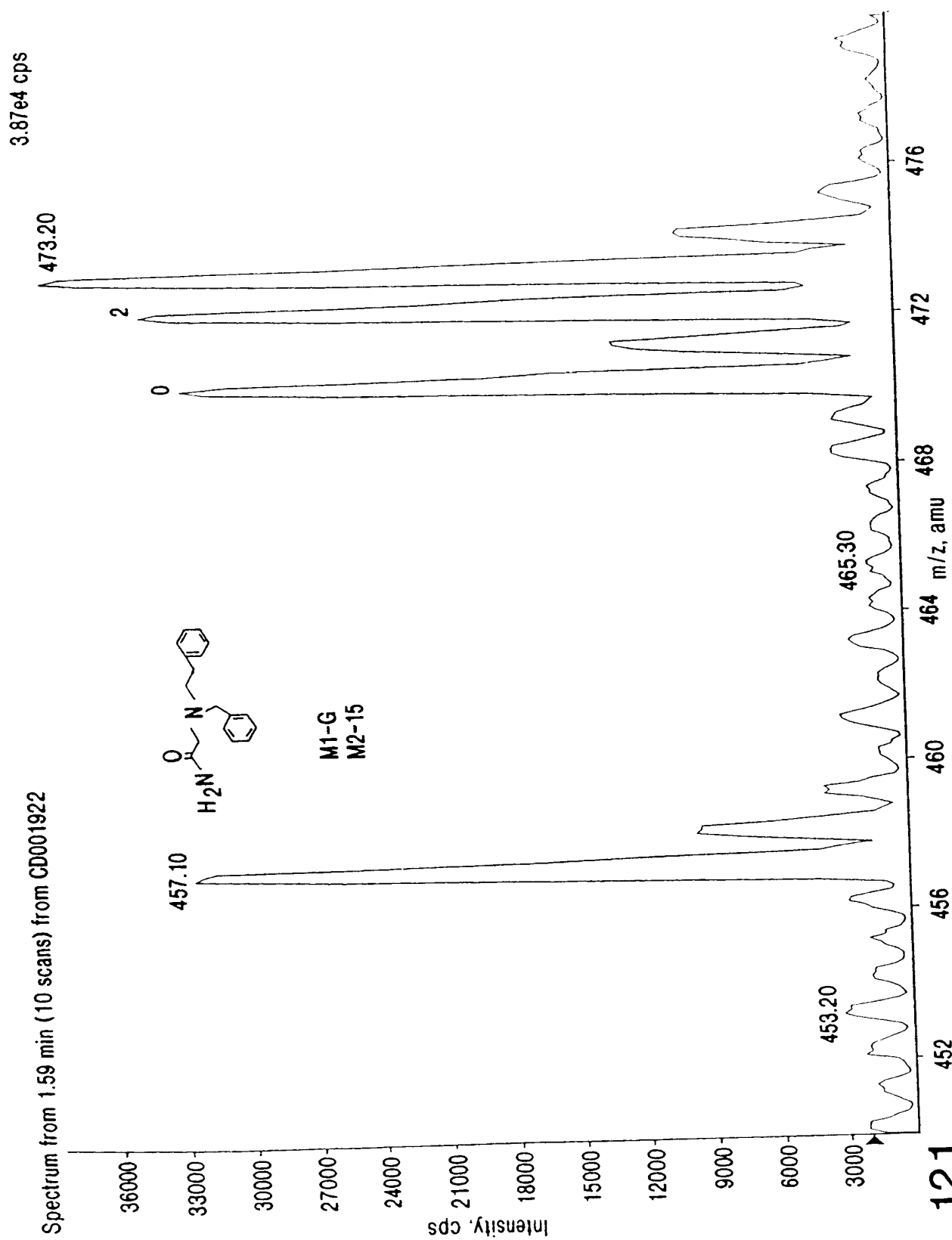


FIG. 121

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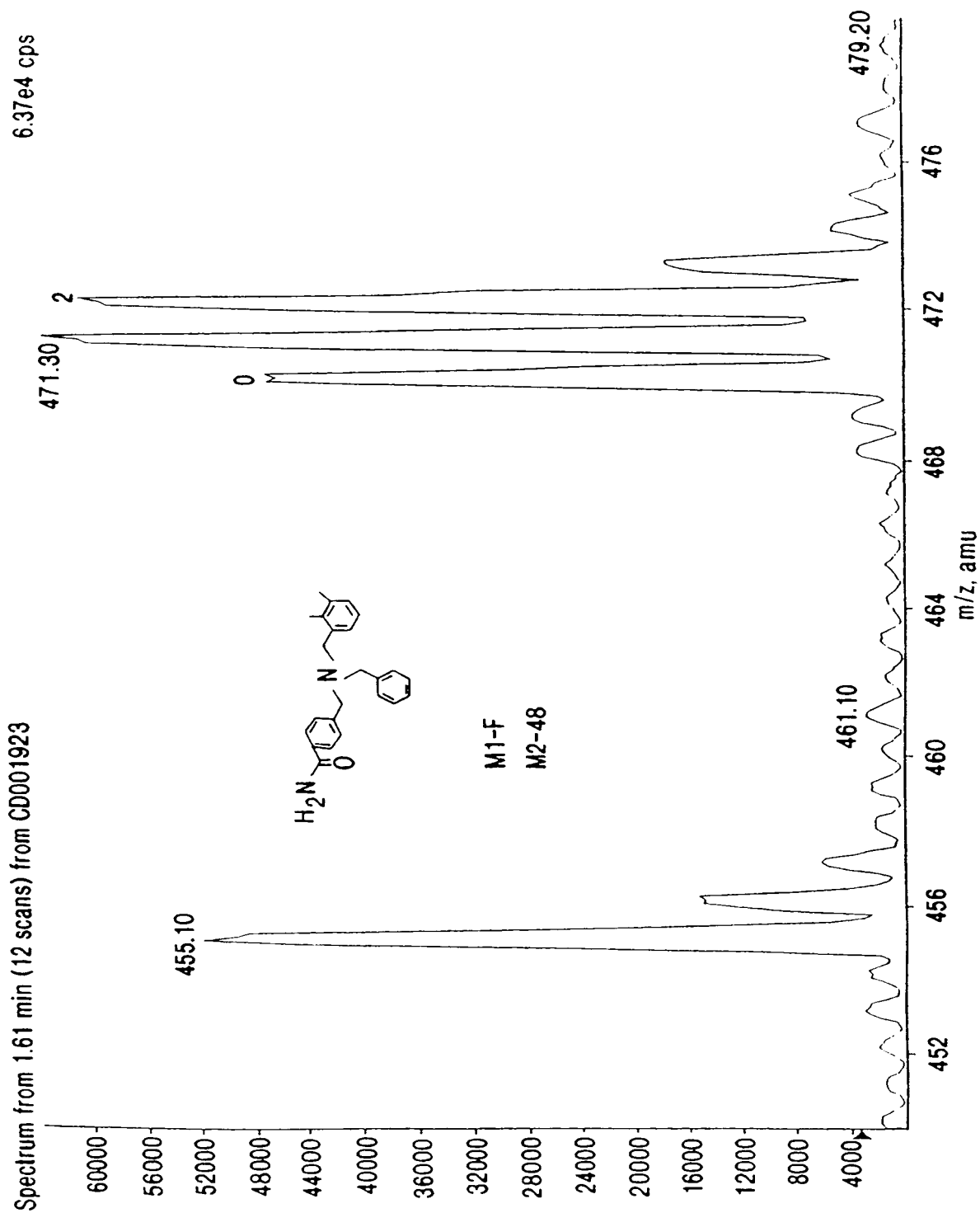


FIG. 122

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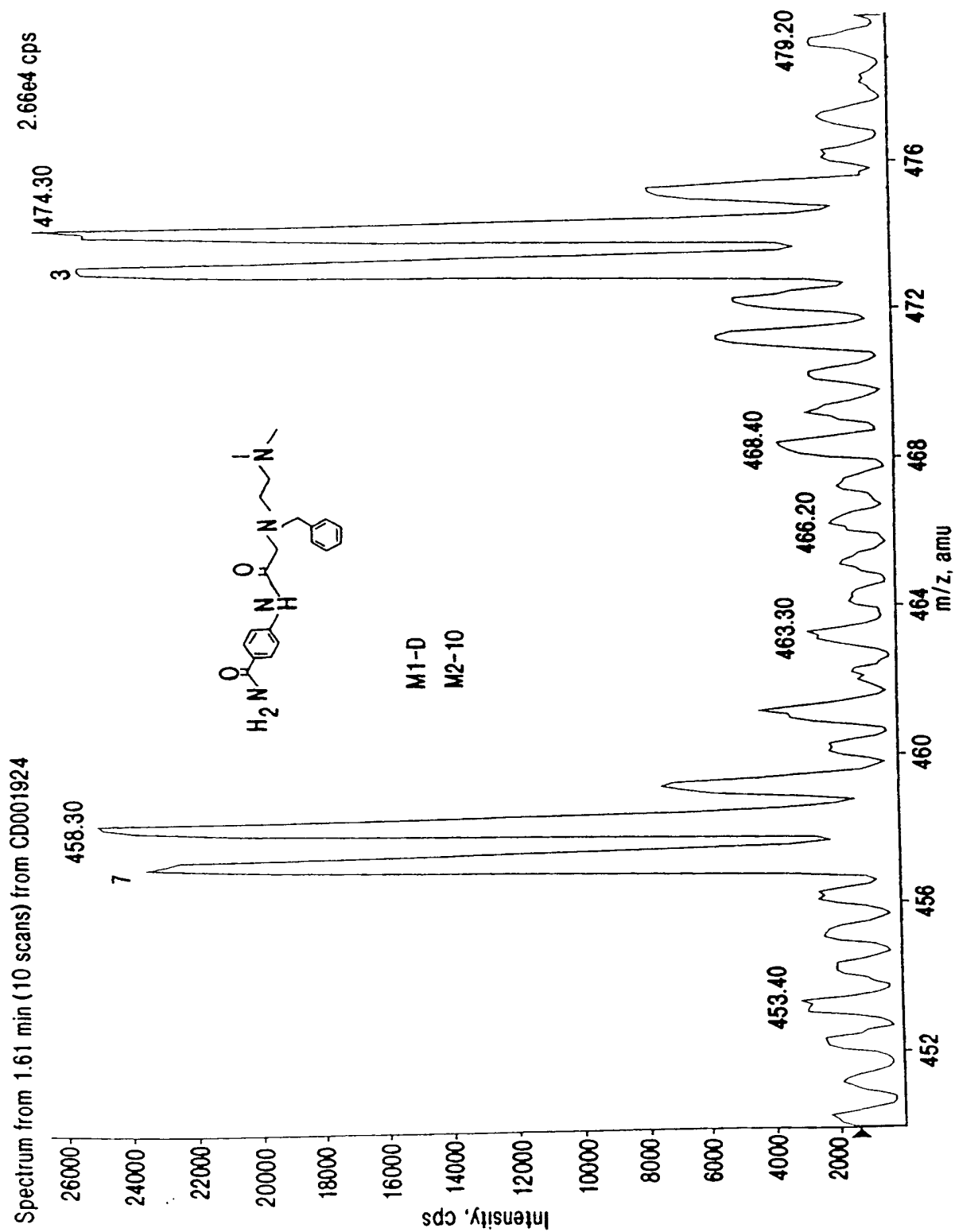


FIG. 123

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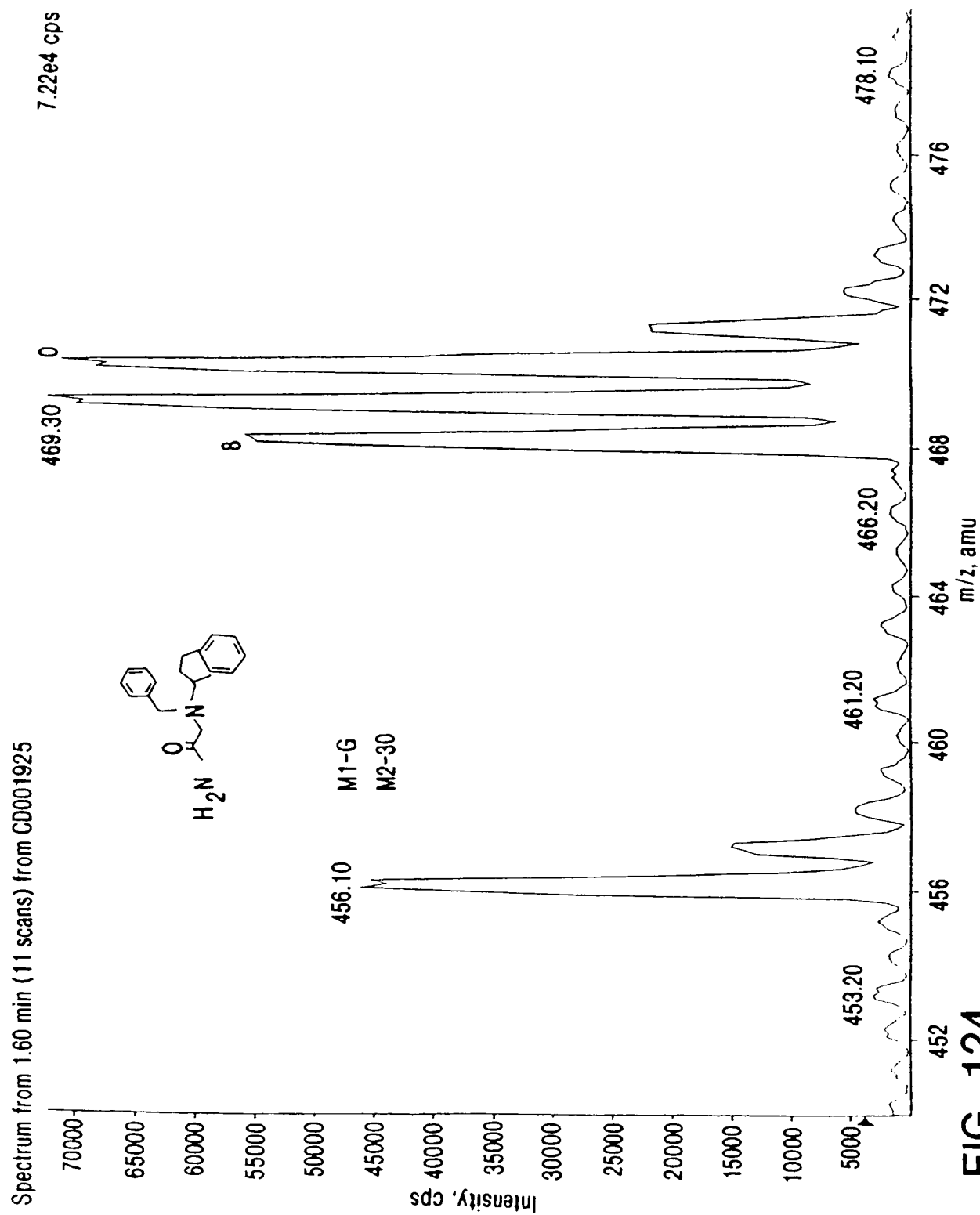


FIG. 124

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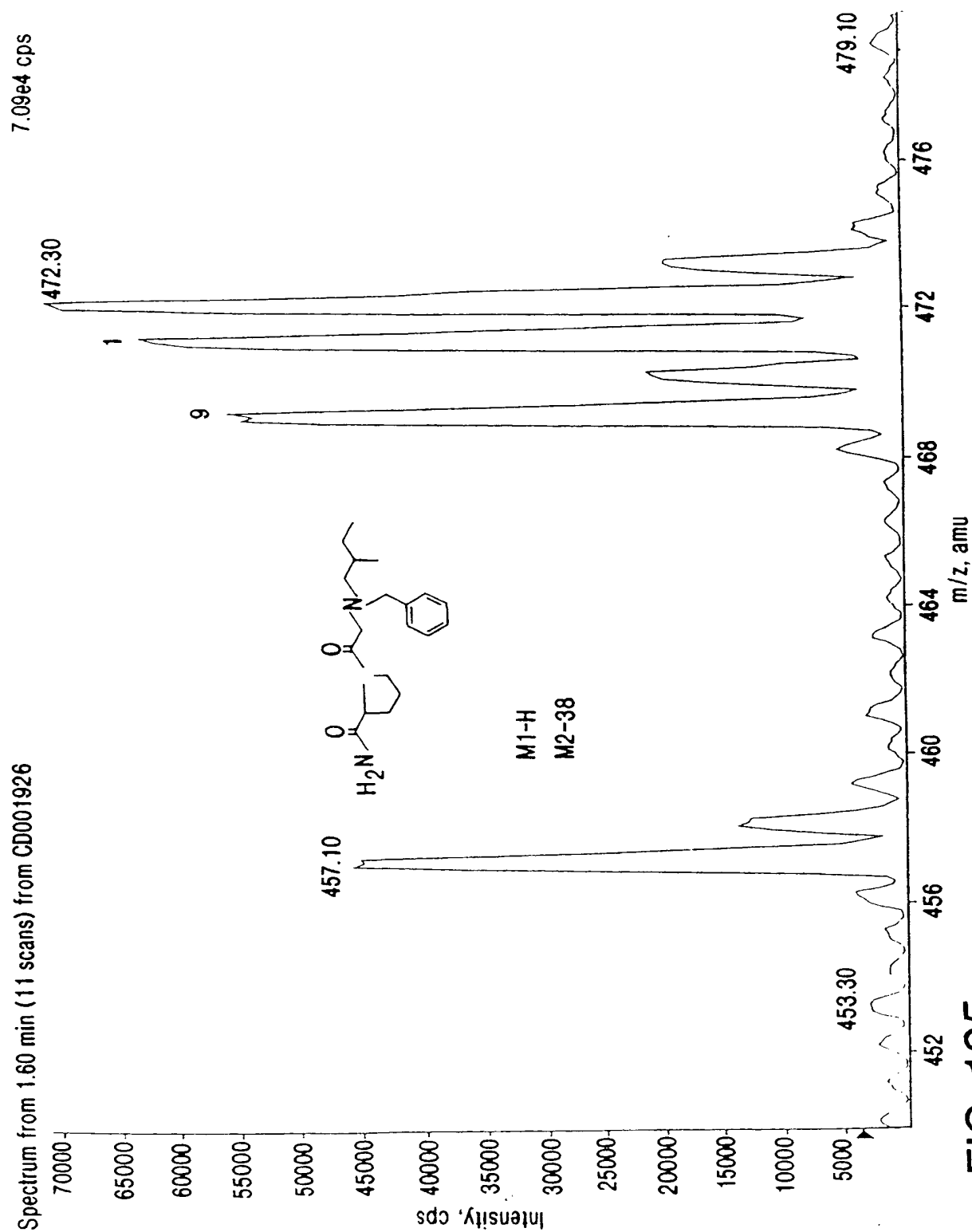


FIG. 125

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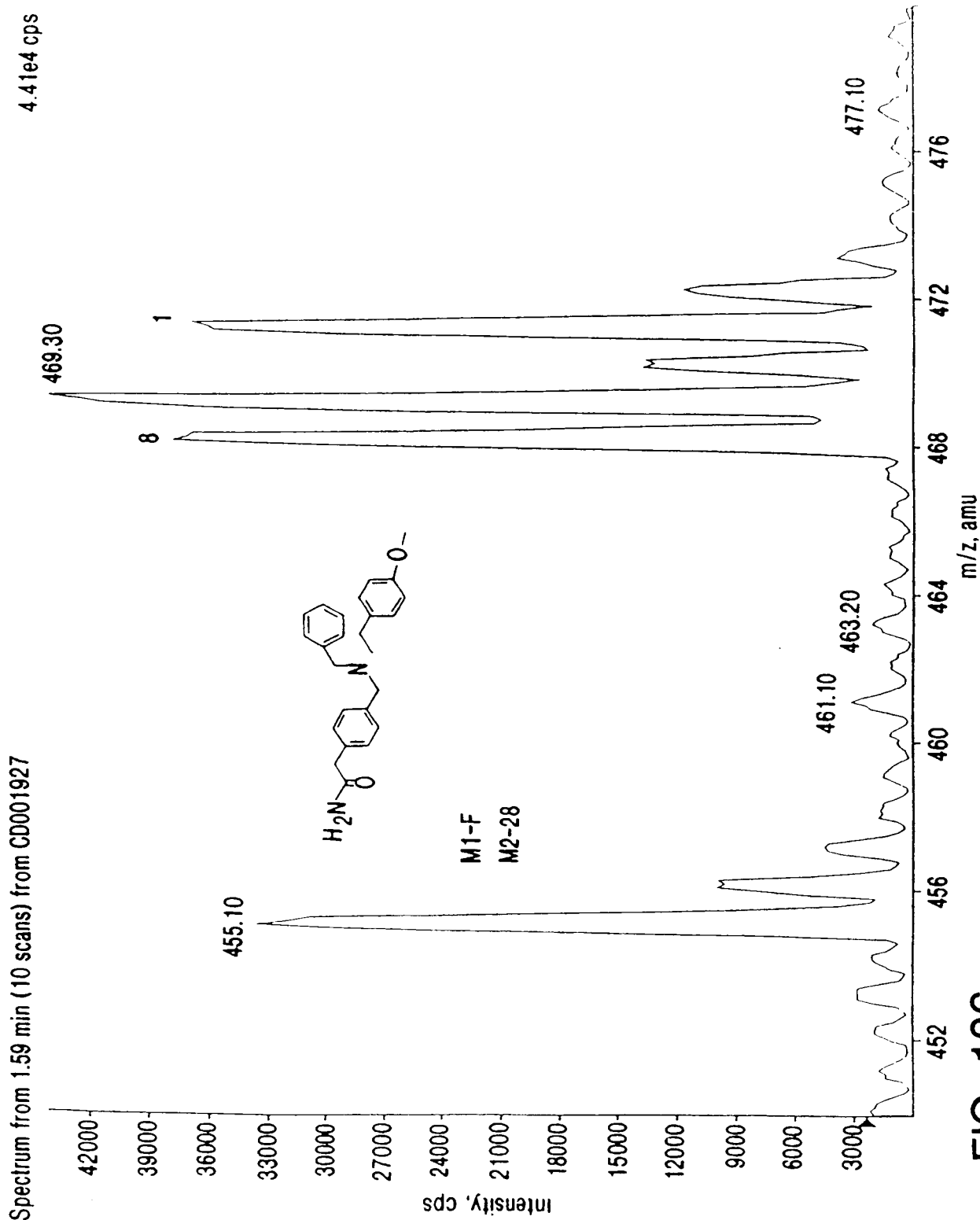


FIG. 126

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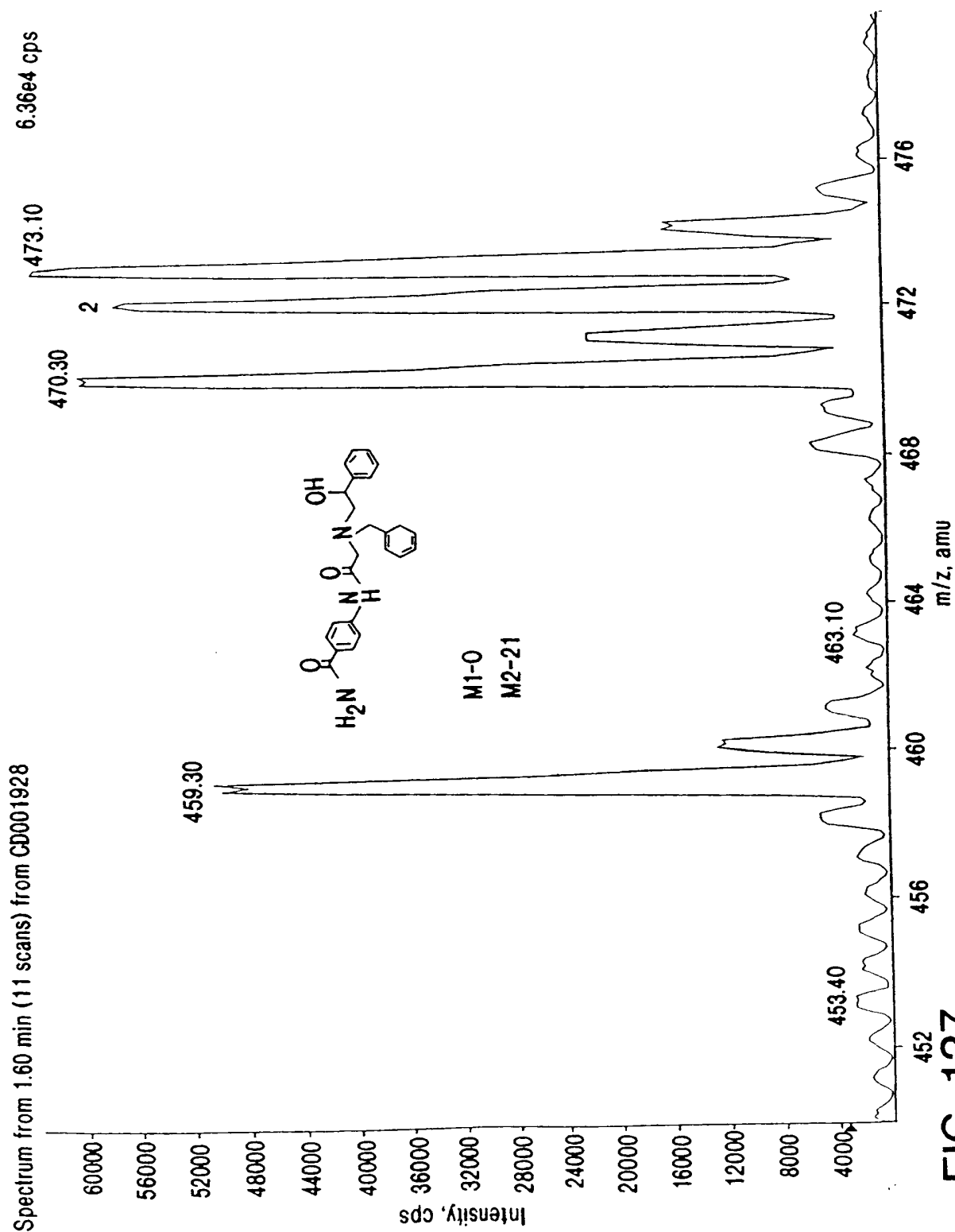


FIG. 127

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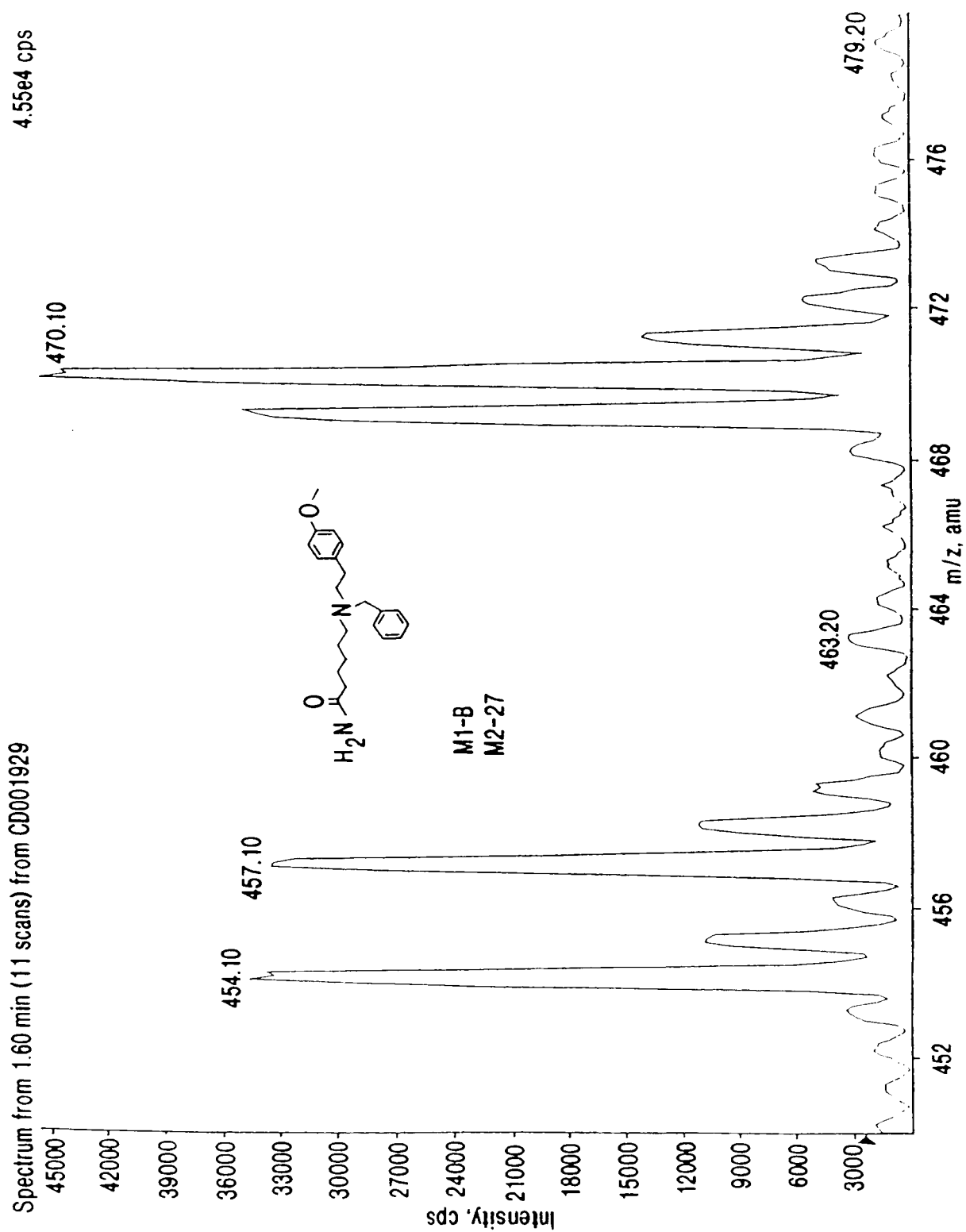


FIG. 128

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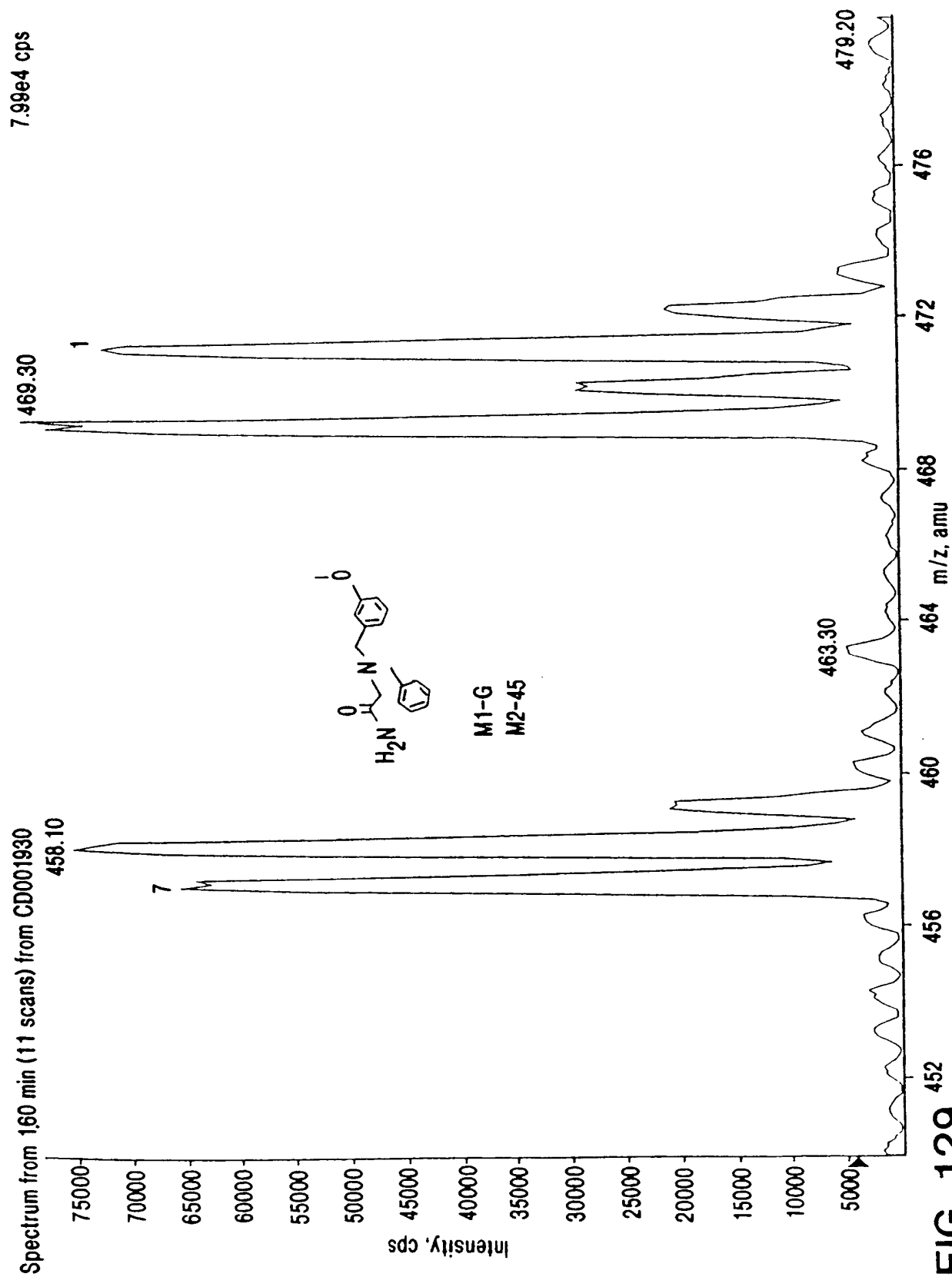


FIG. 129

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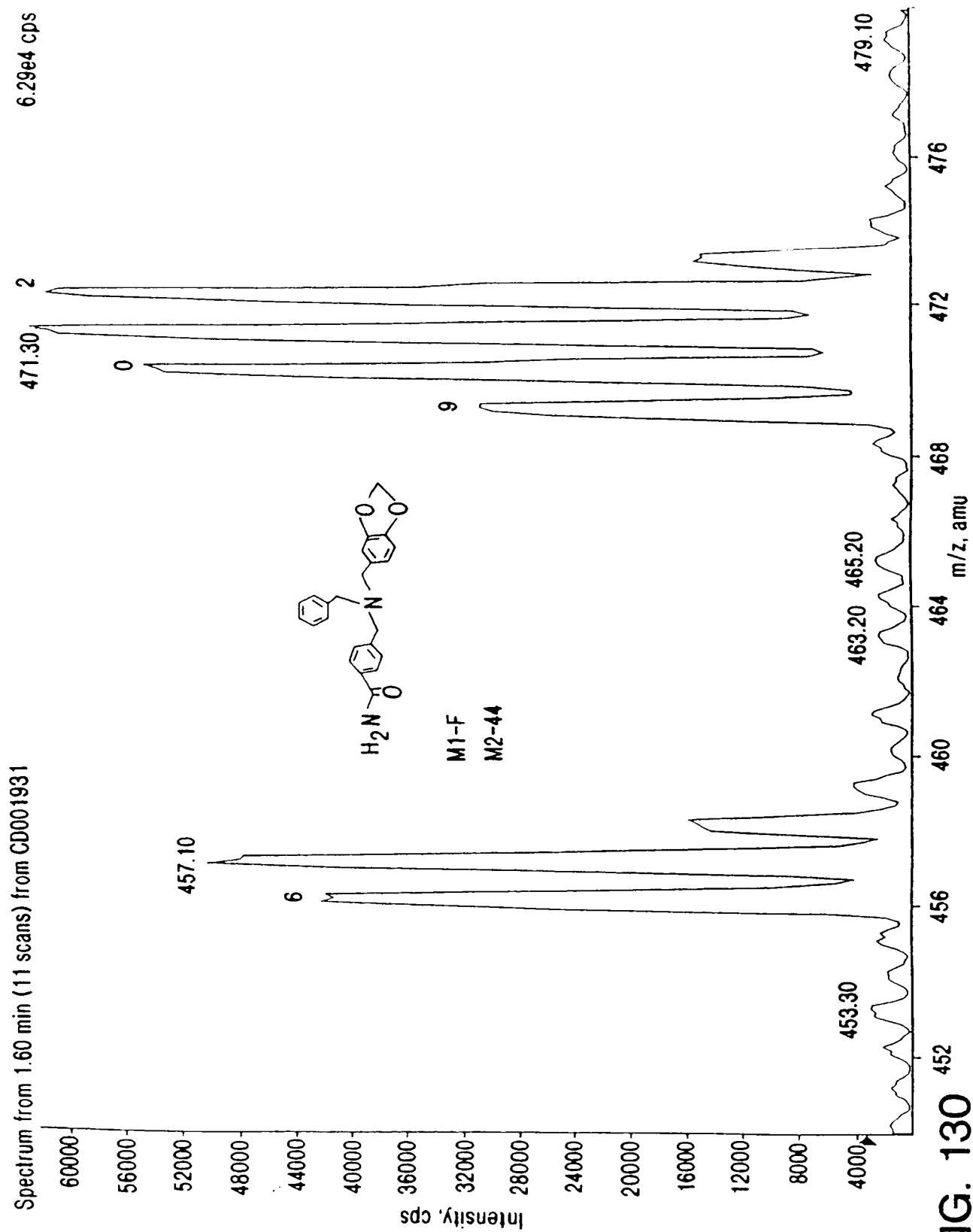
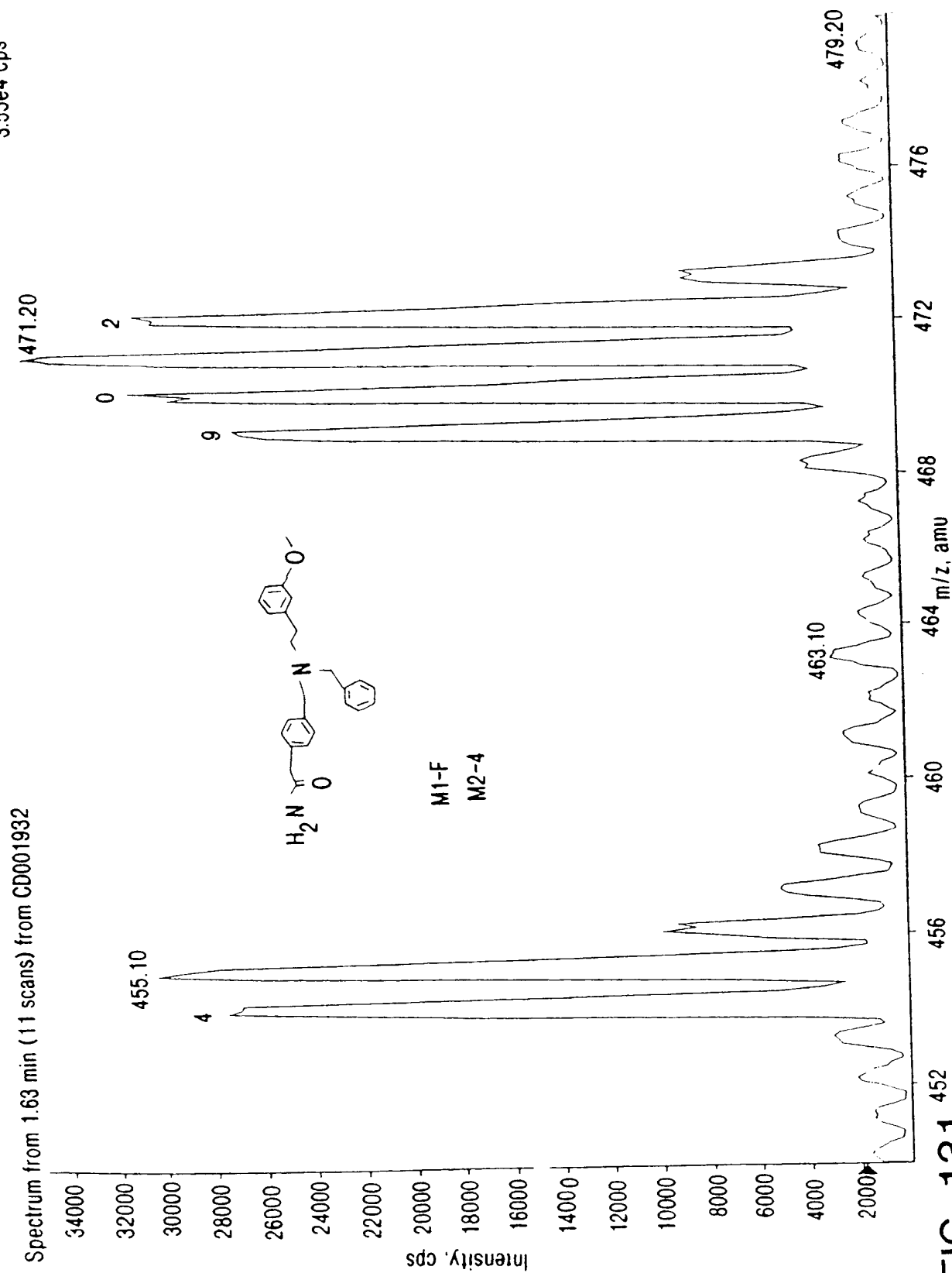


FIG. 130

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3.55e4 cps



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2.85e4 cps

Spectrum from 1.63 min (11 scans) from CD001933

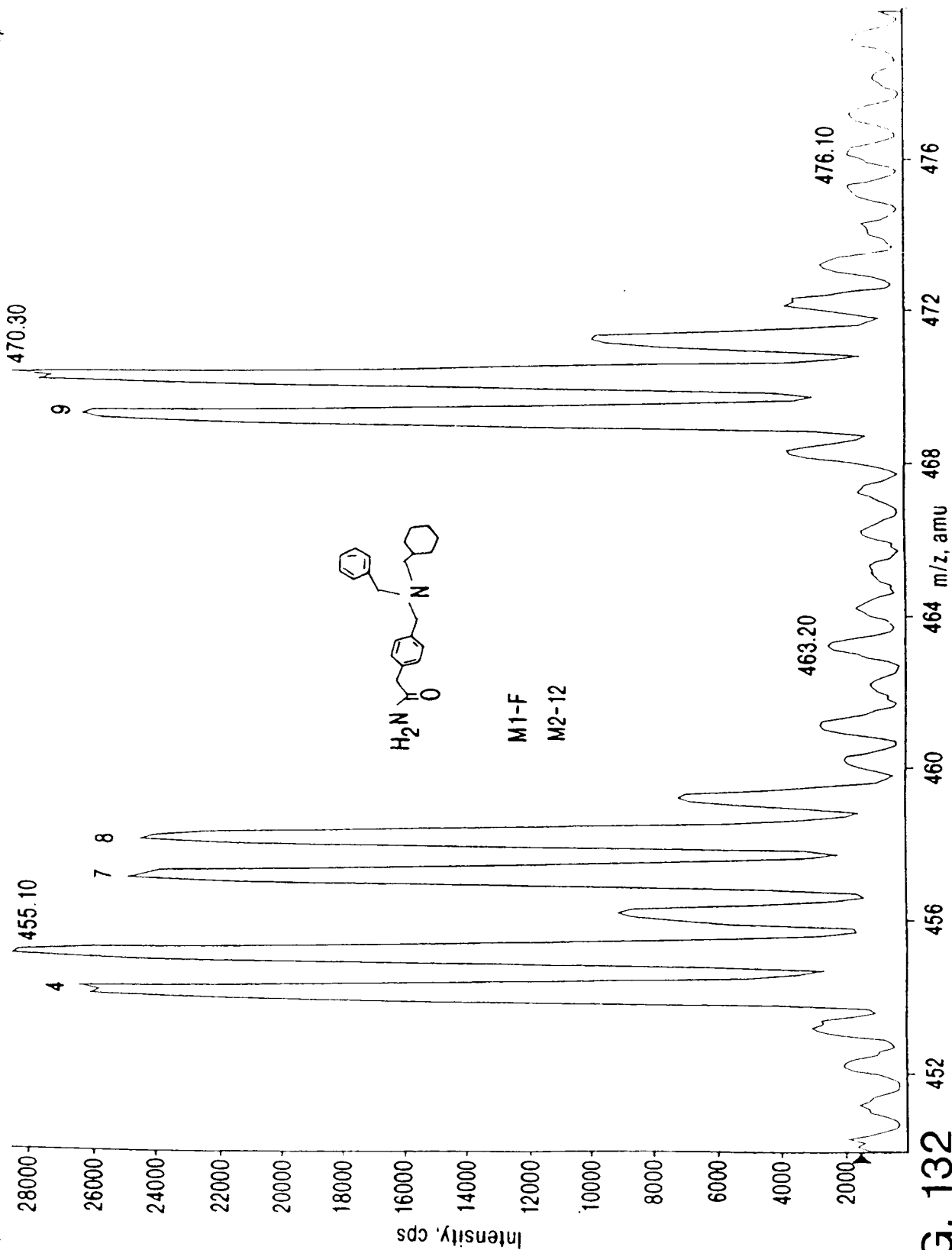


FIG. 132

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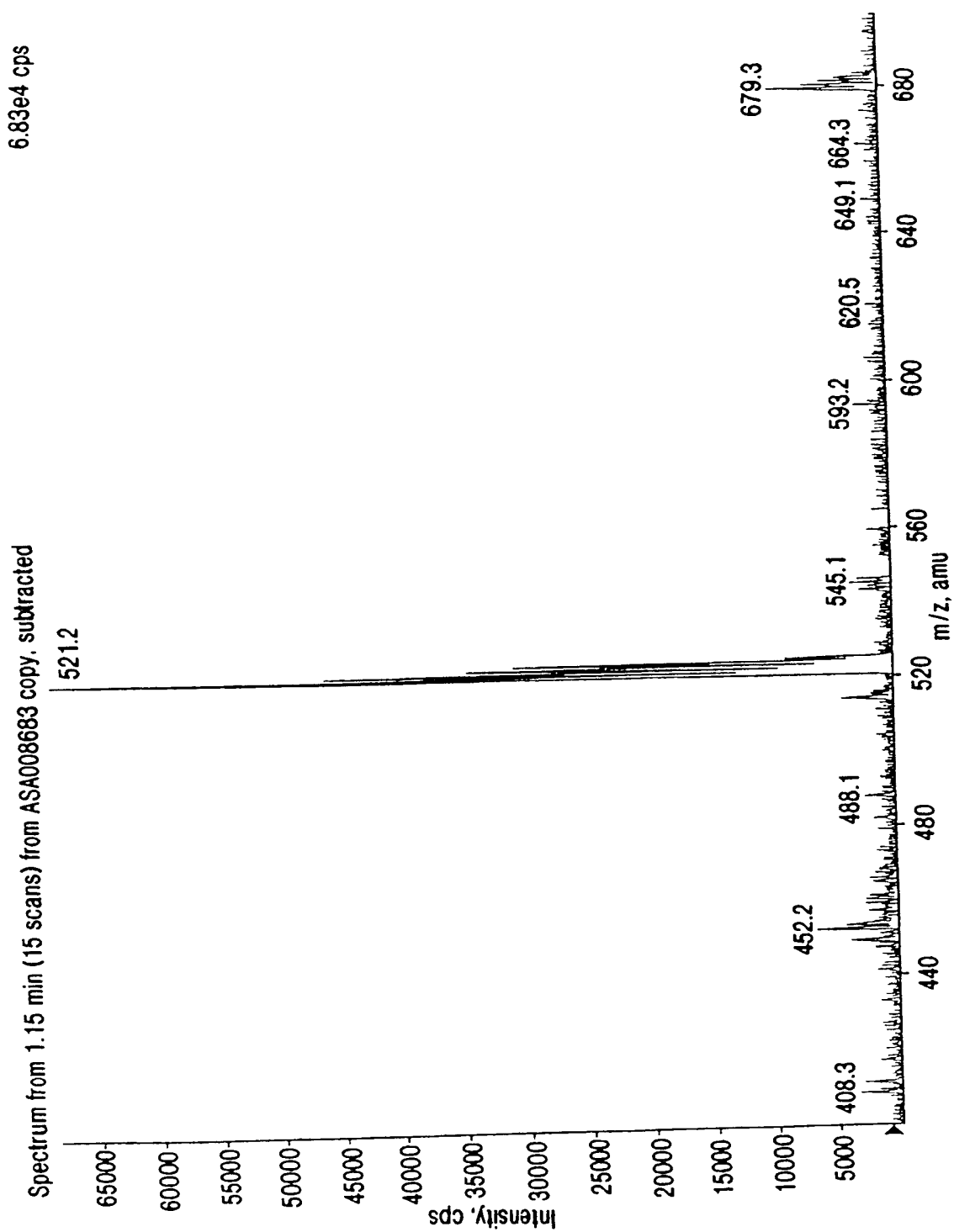


FIG. 133

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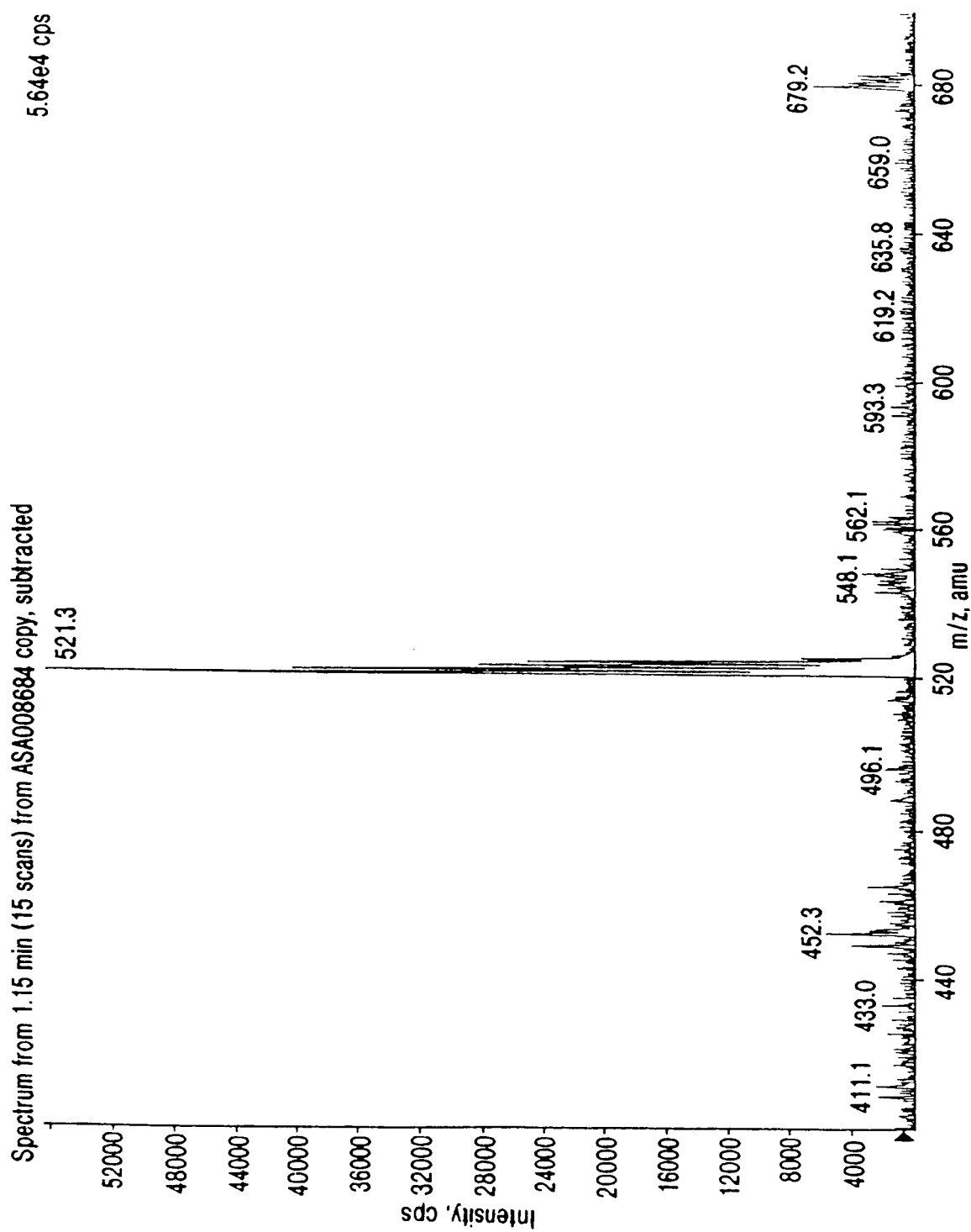


FIG. 134

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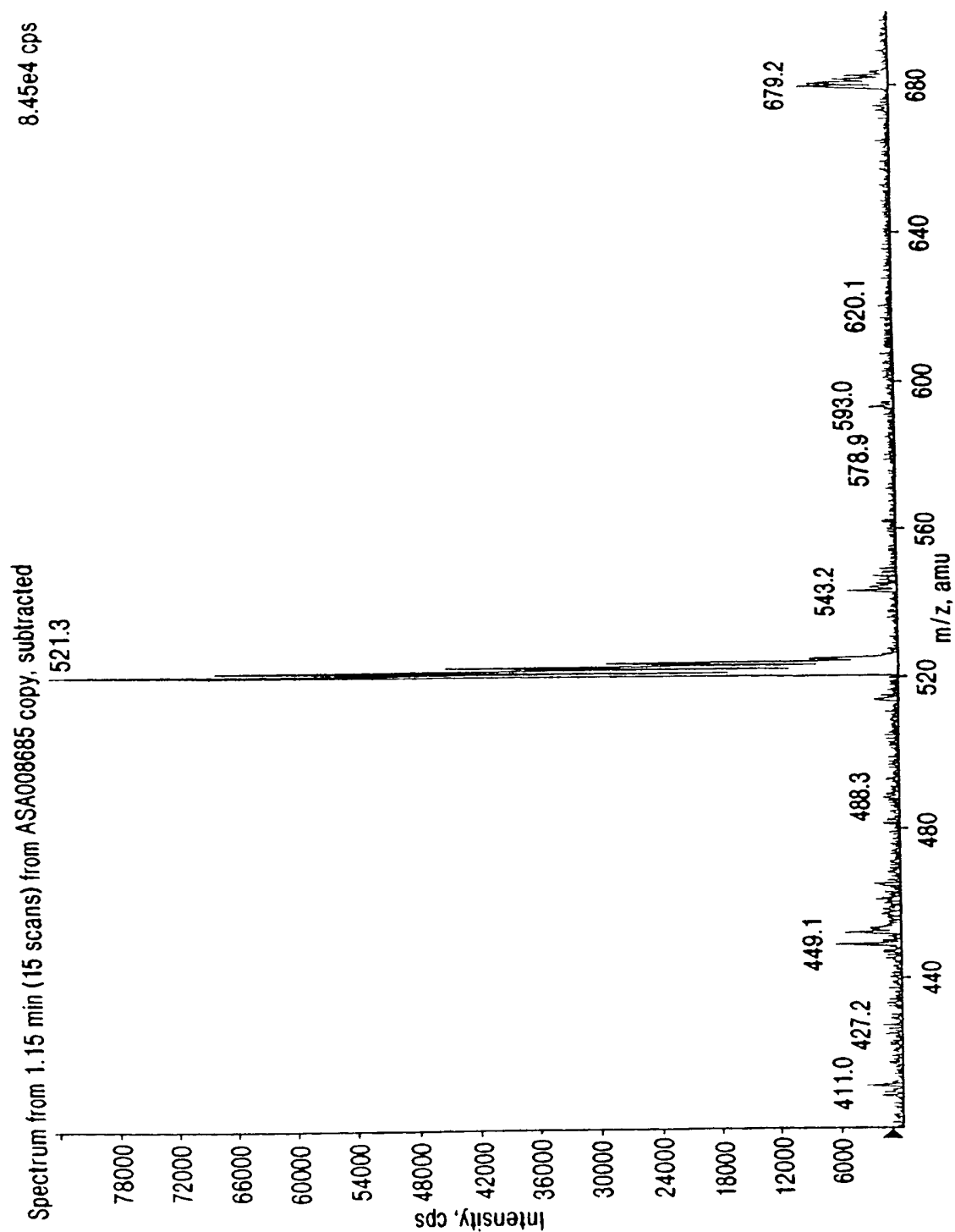


FIG. 135

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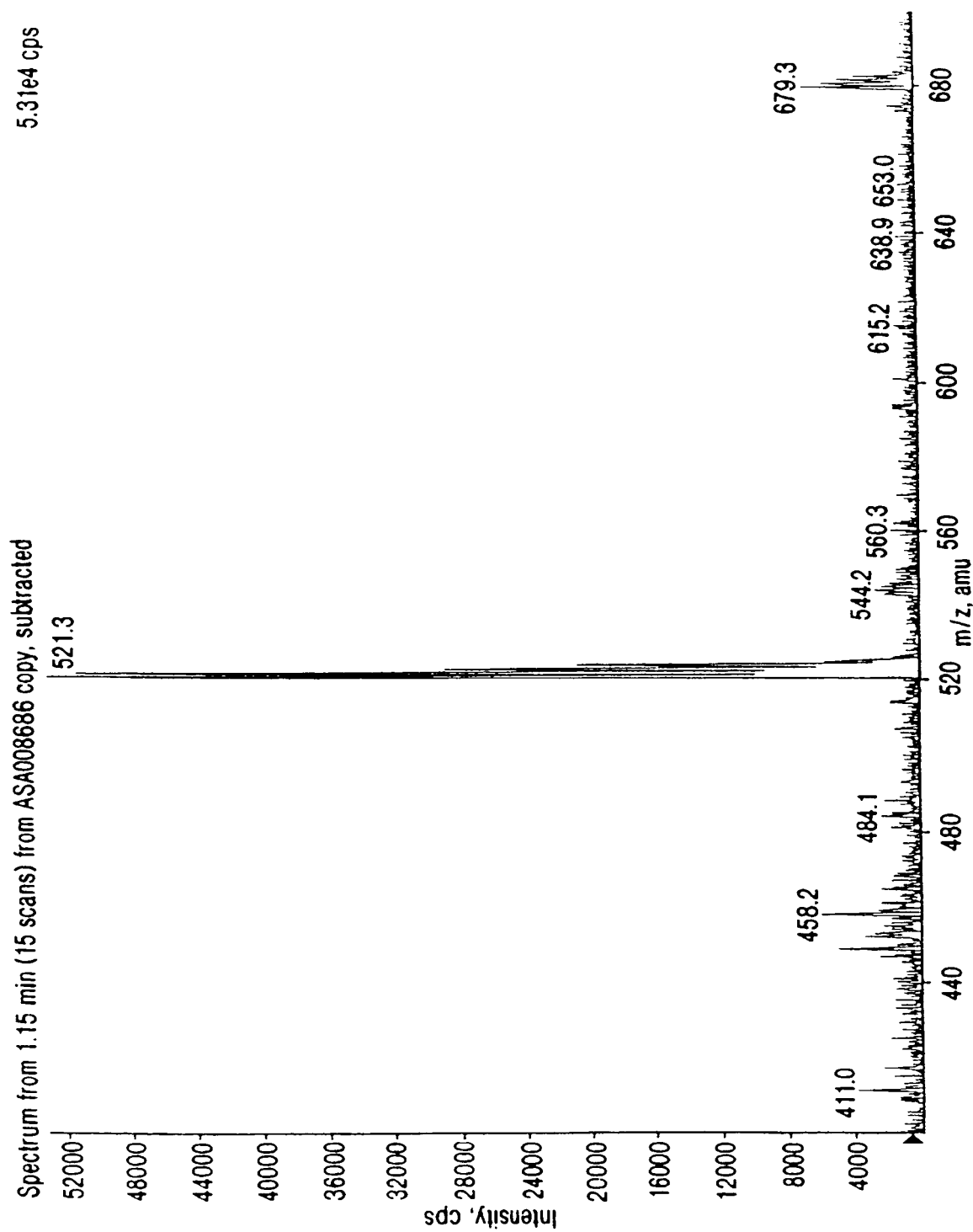


FIG. 136

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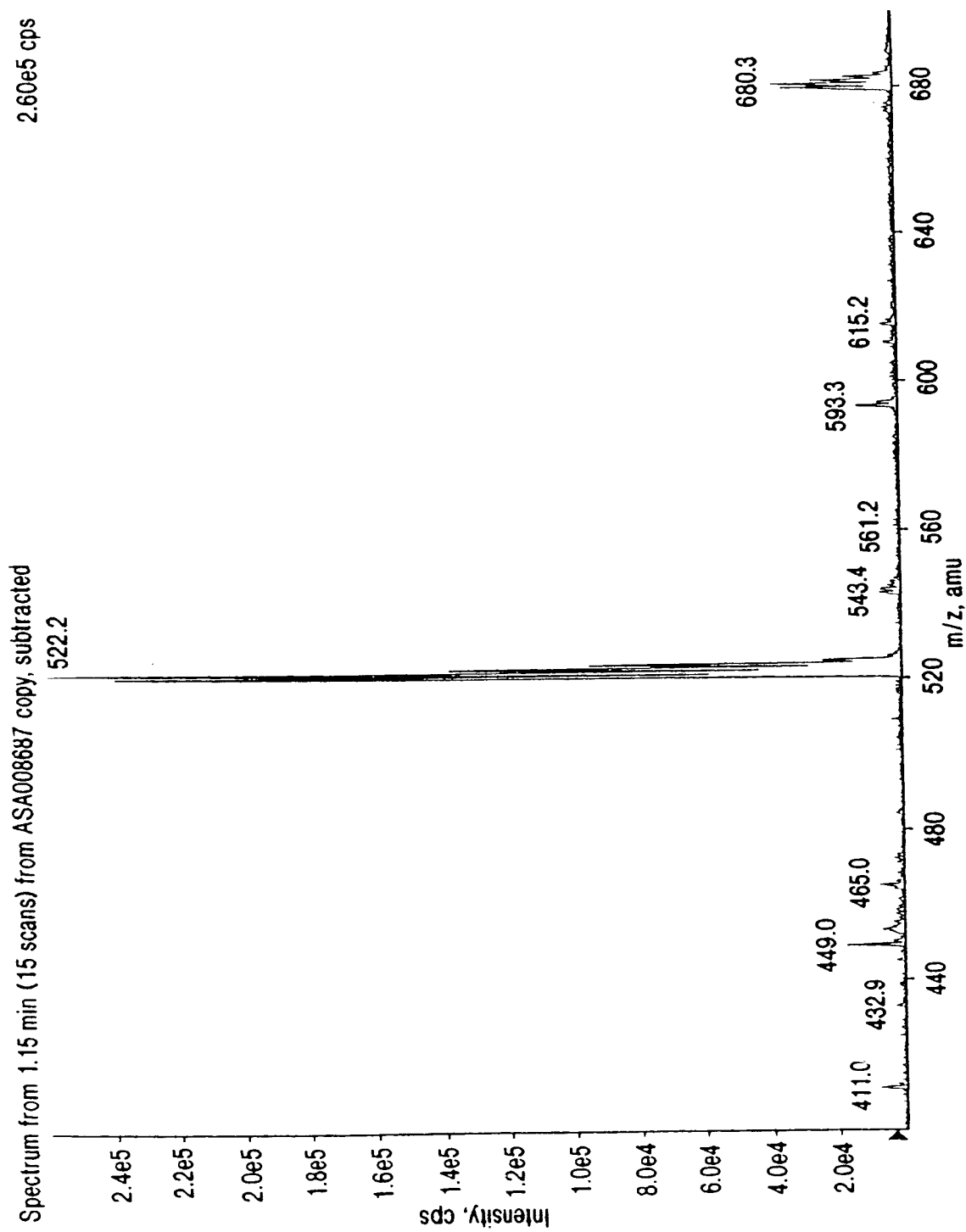


FIG. 137

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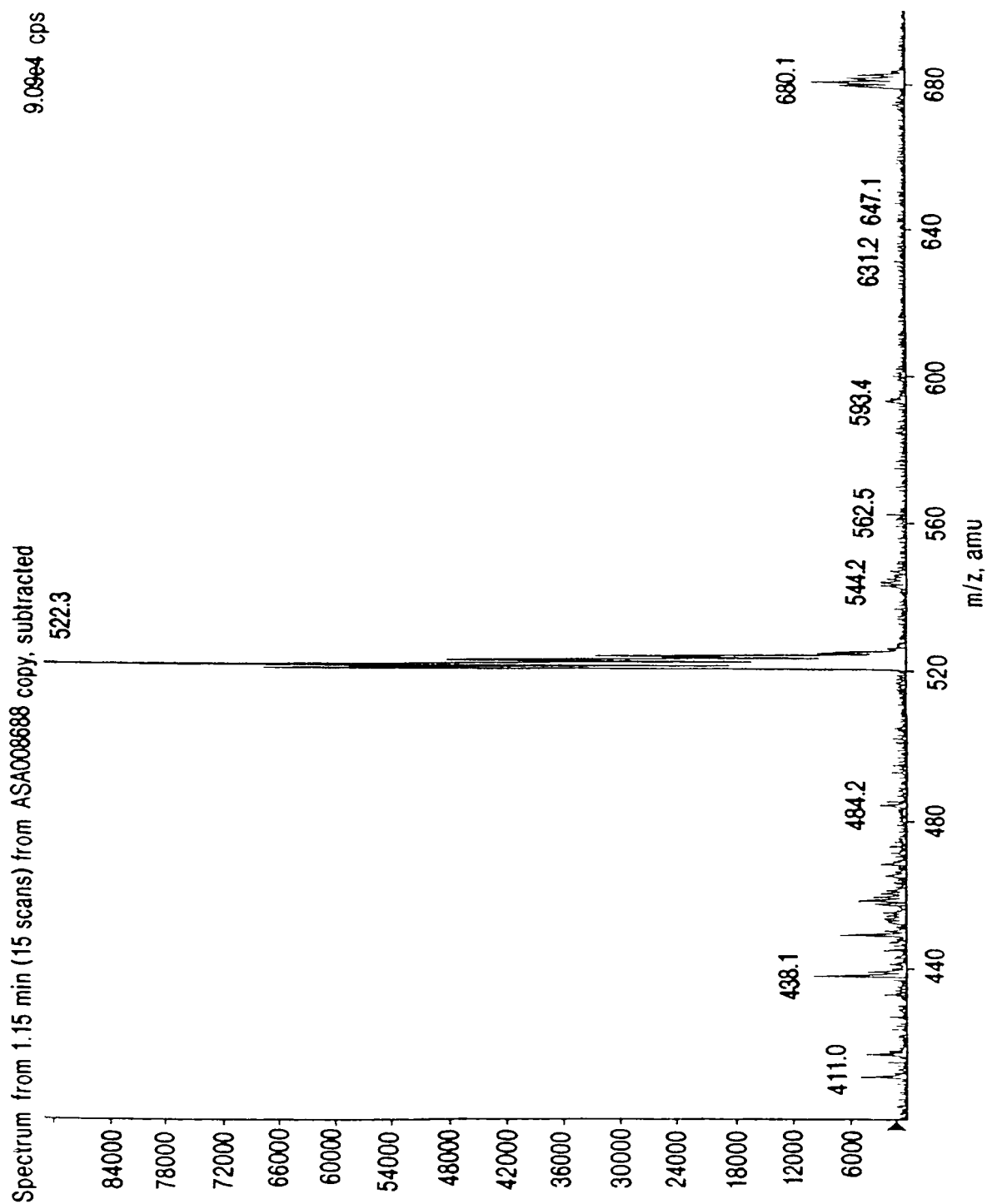


FIG. 138

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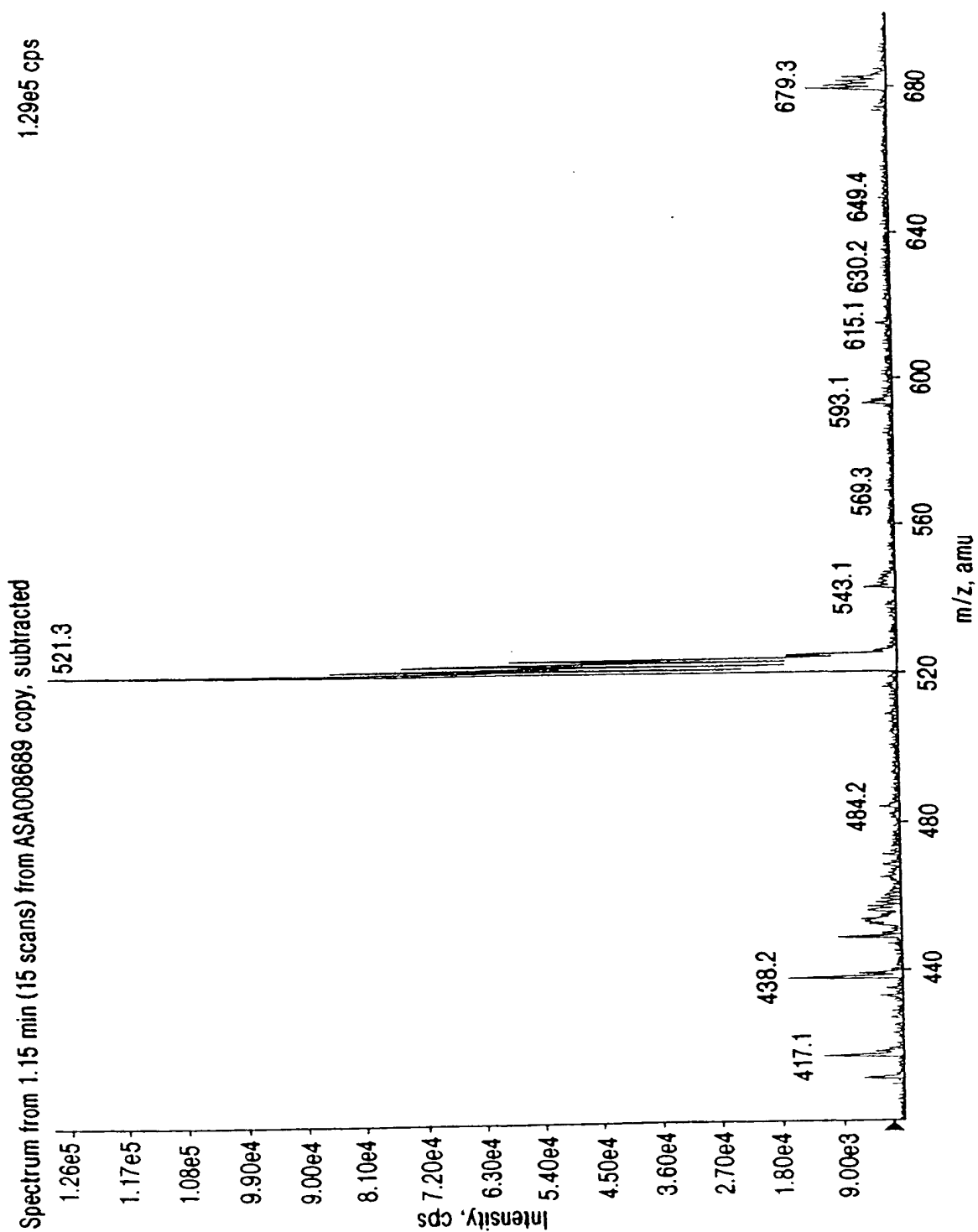


FIG. 139

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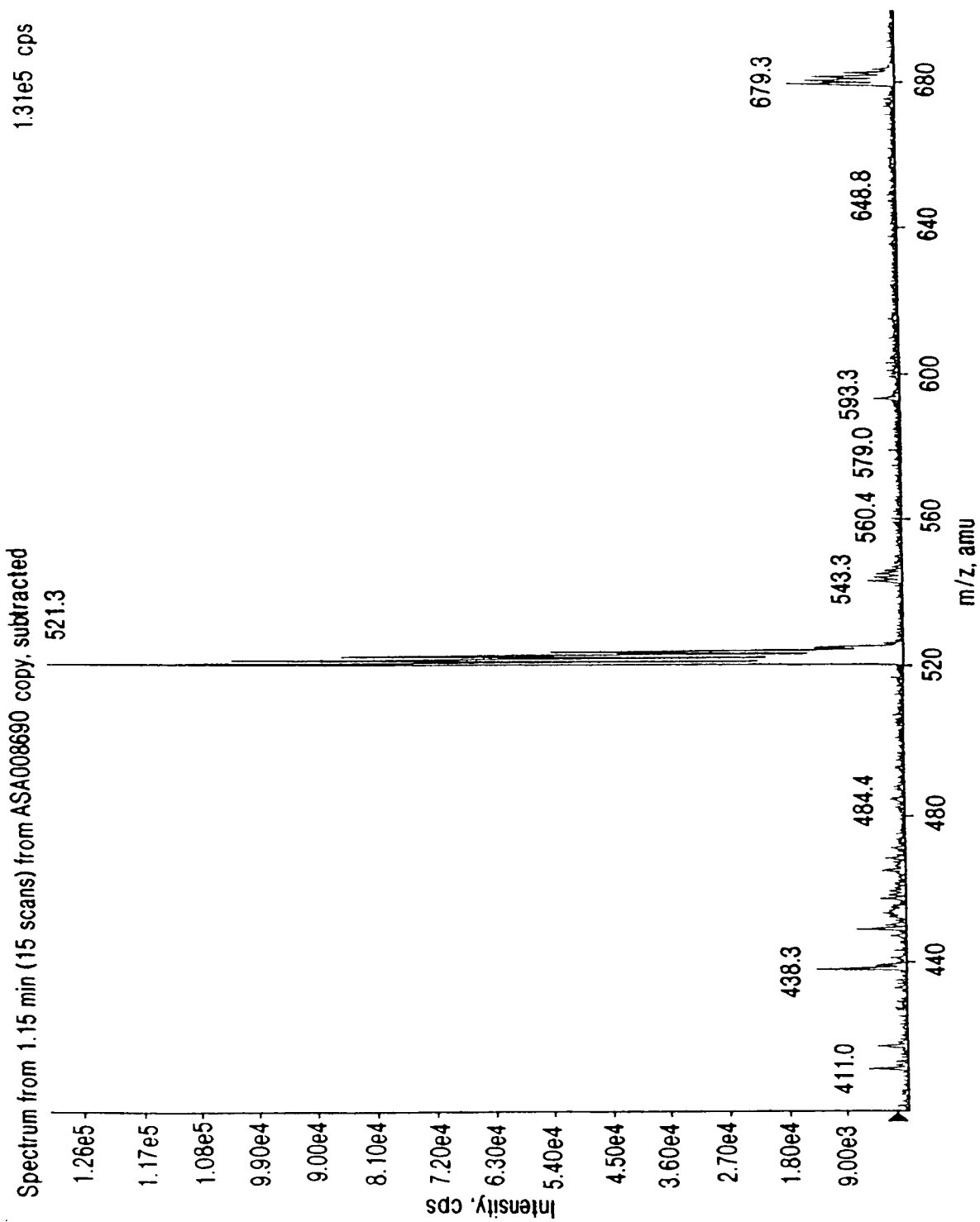


FIG. 140

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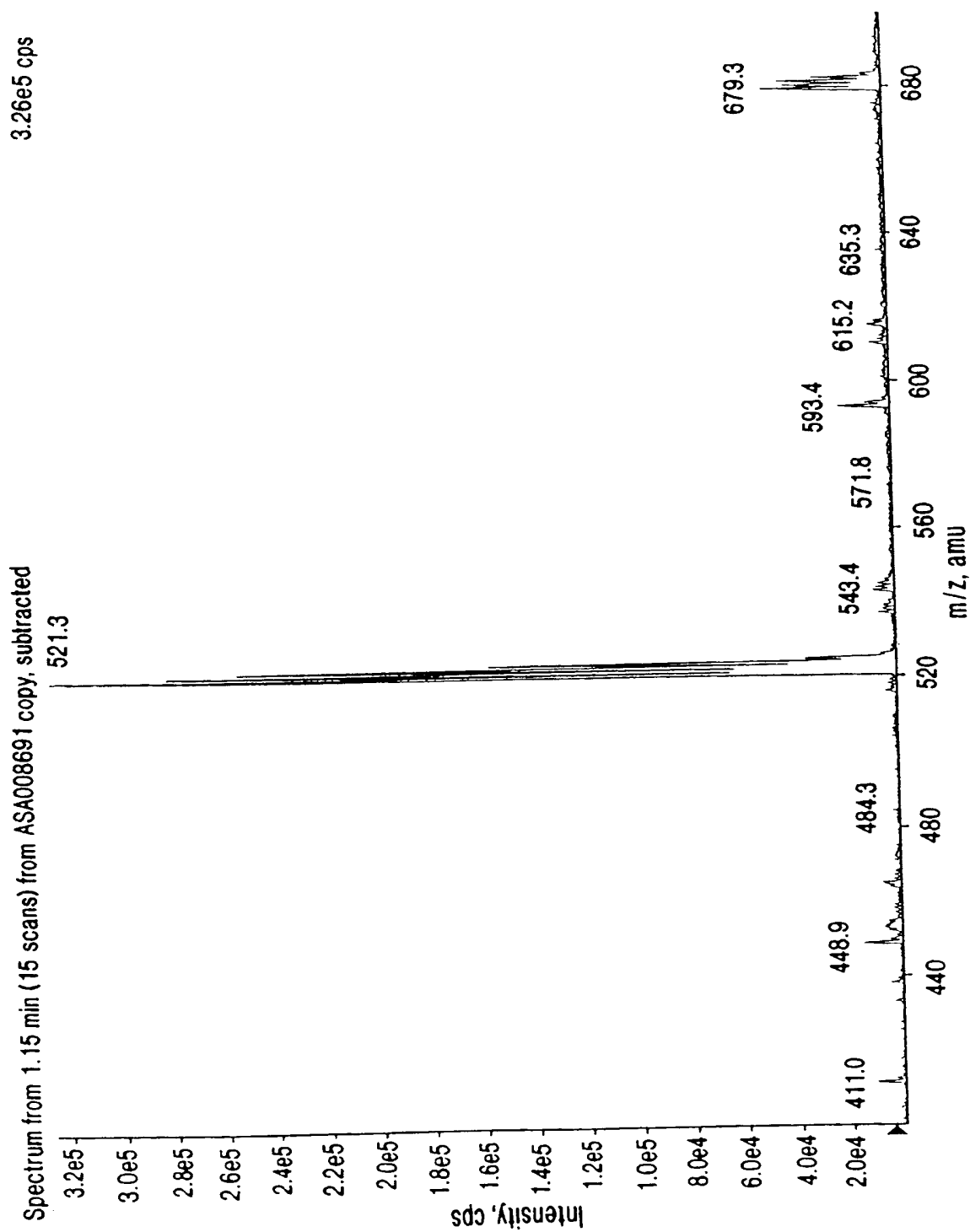


FIG. 141

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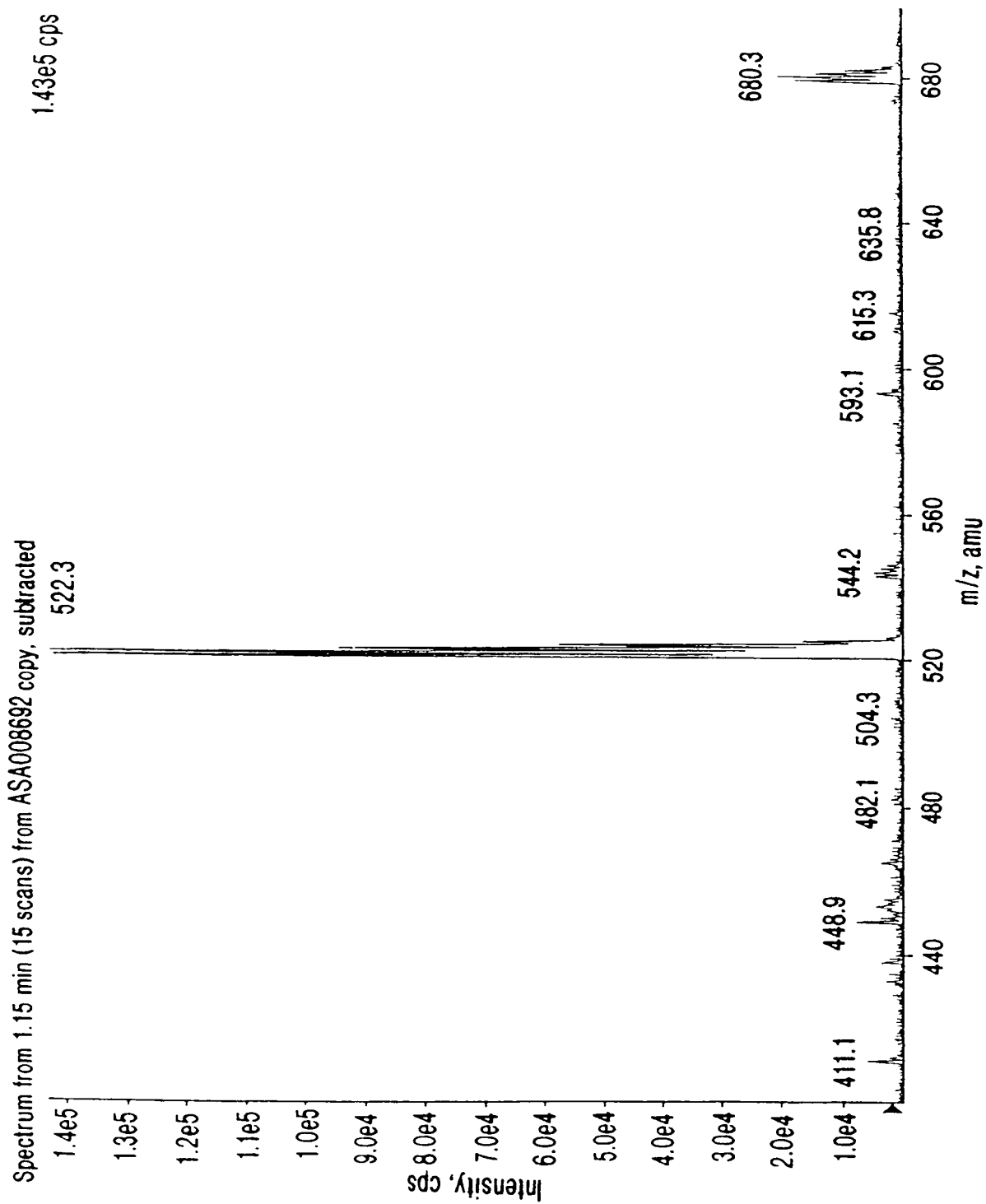


FIG. 142

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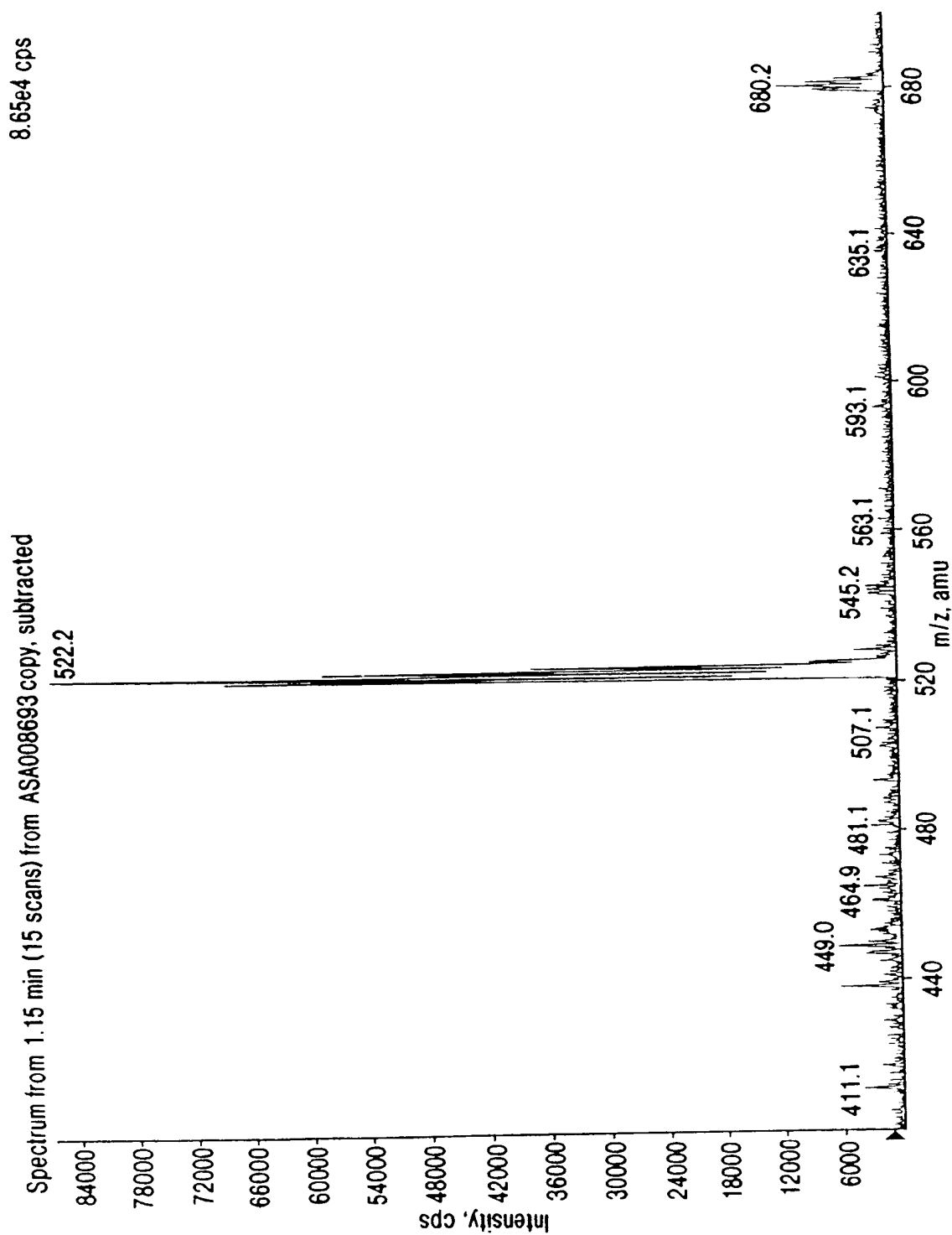


FIG. 143

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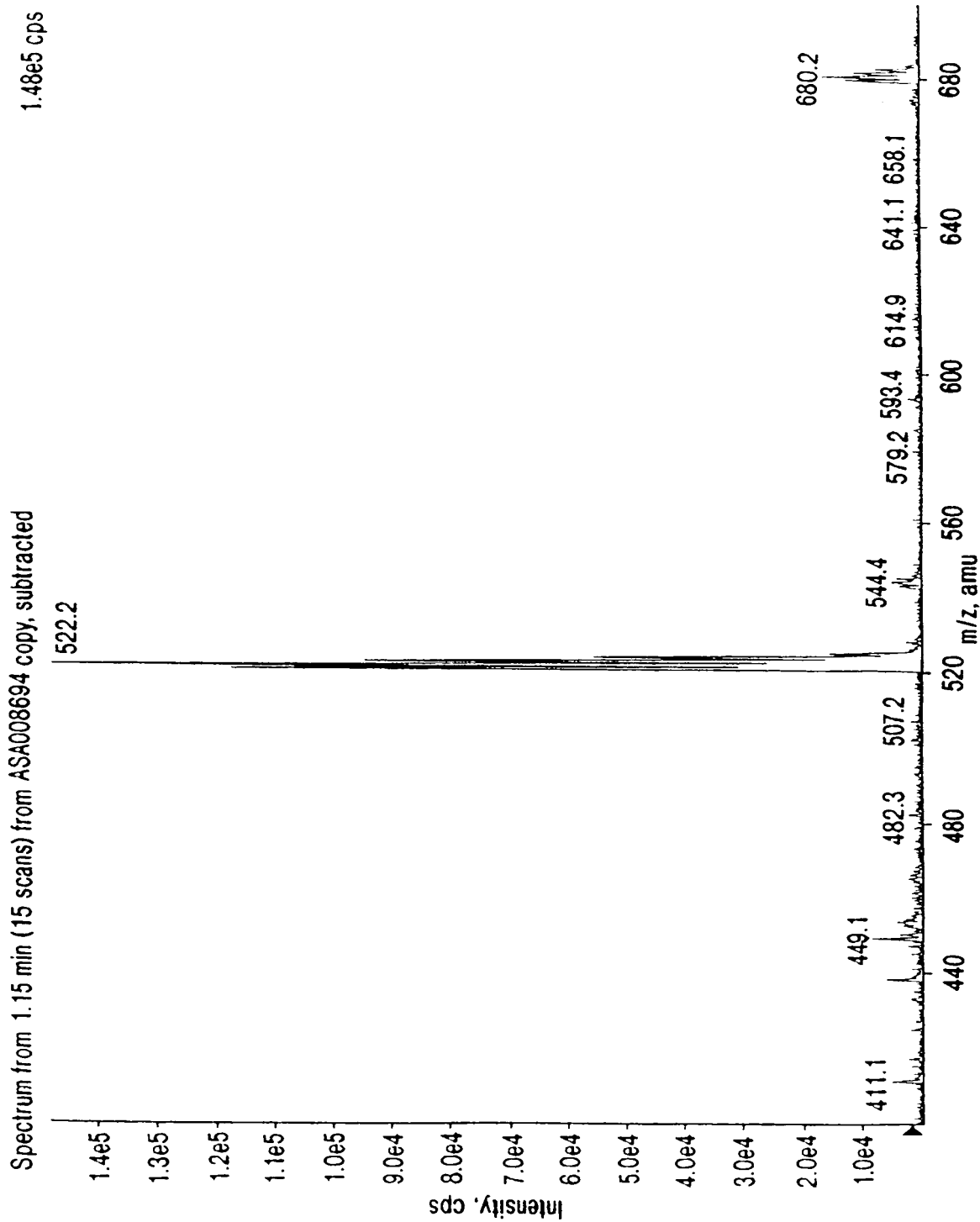


FIG. 144

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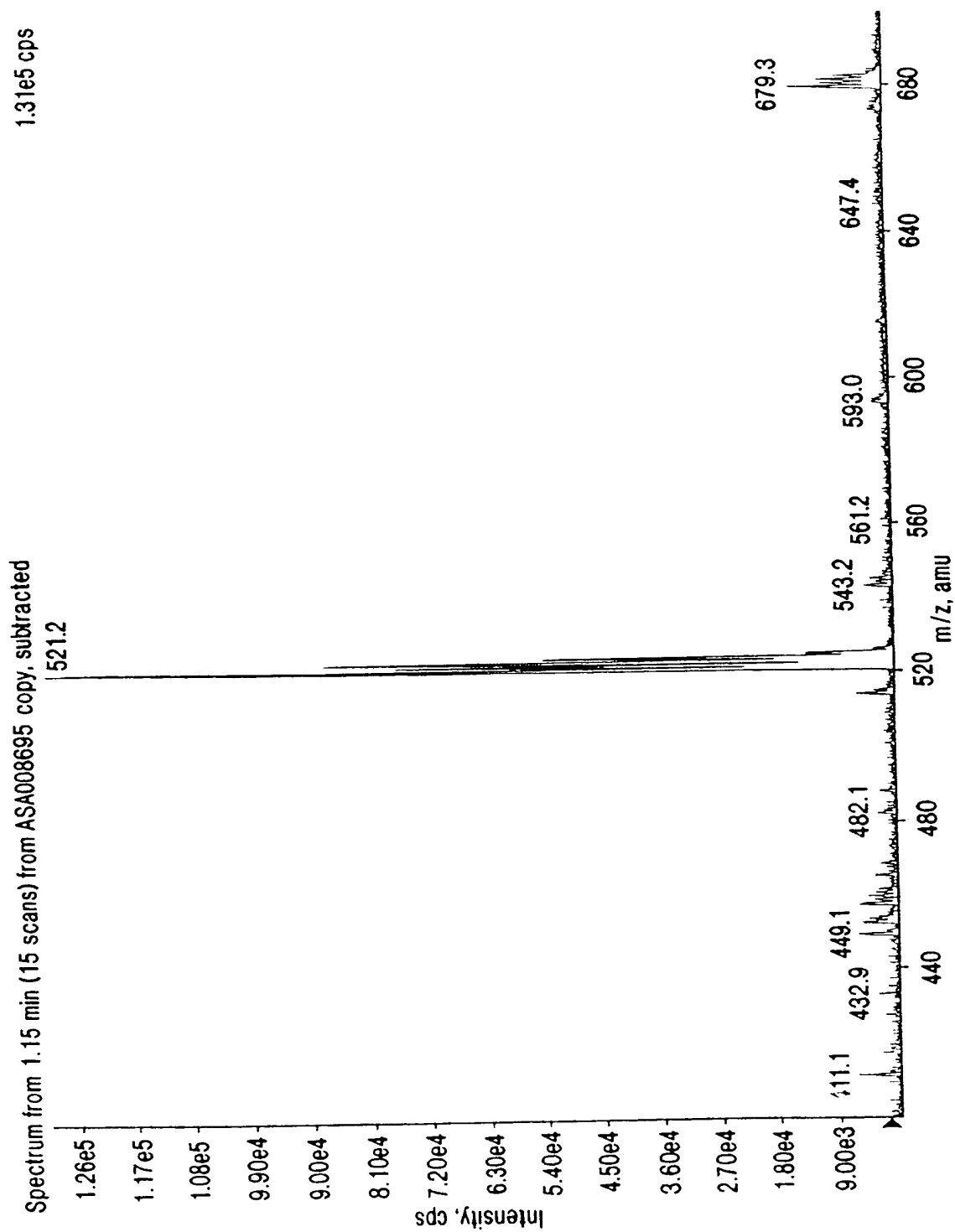


FIG. 145

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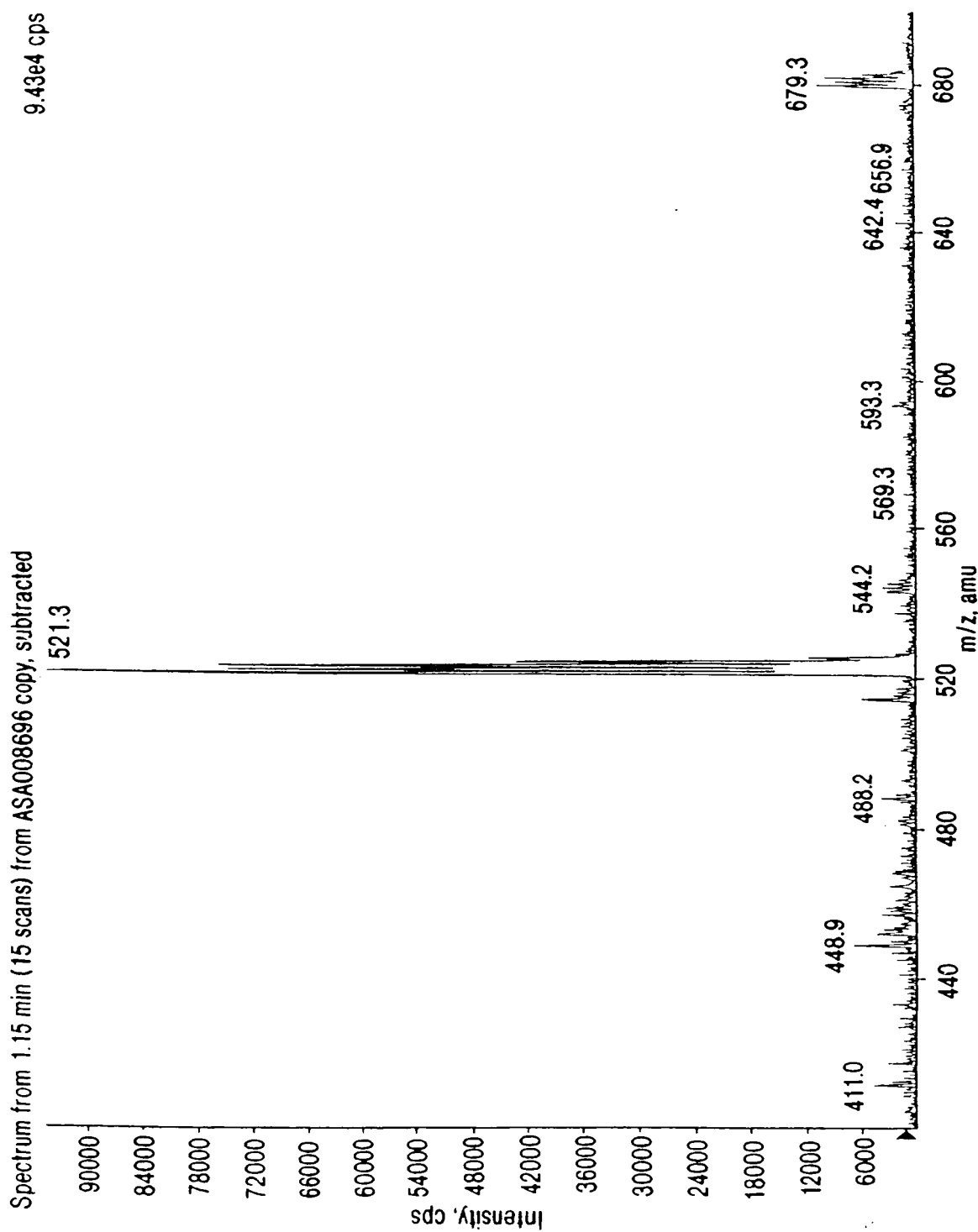


FIG. 146

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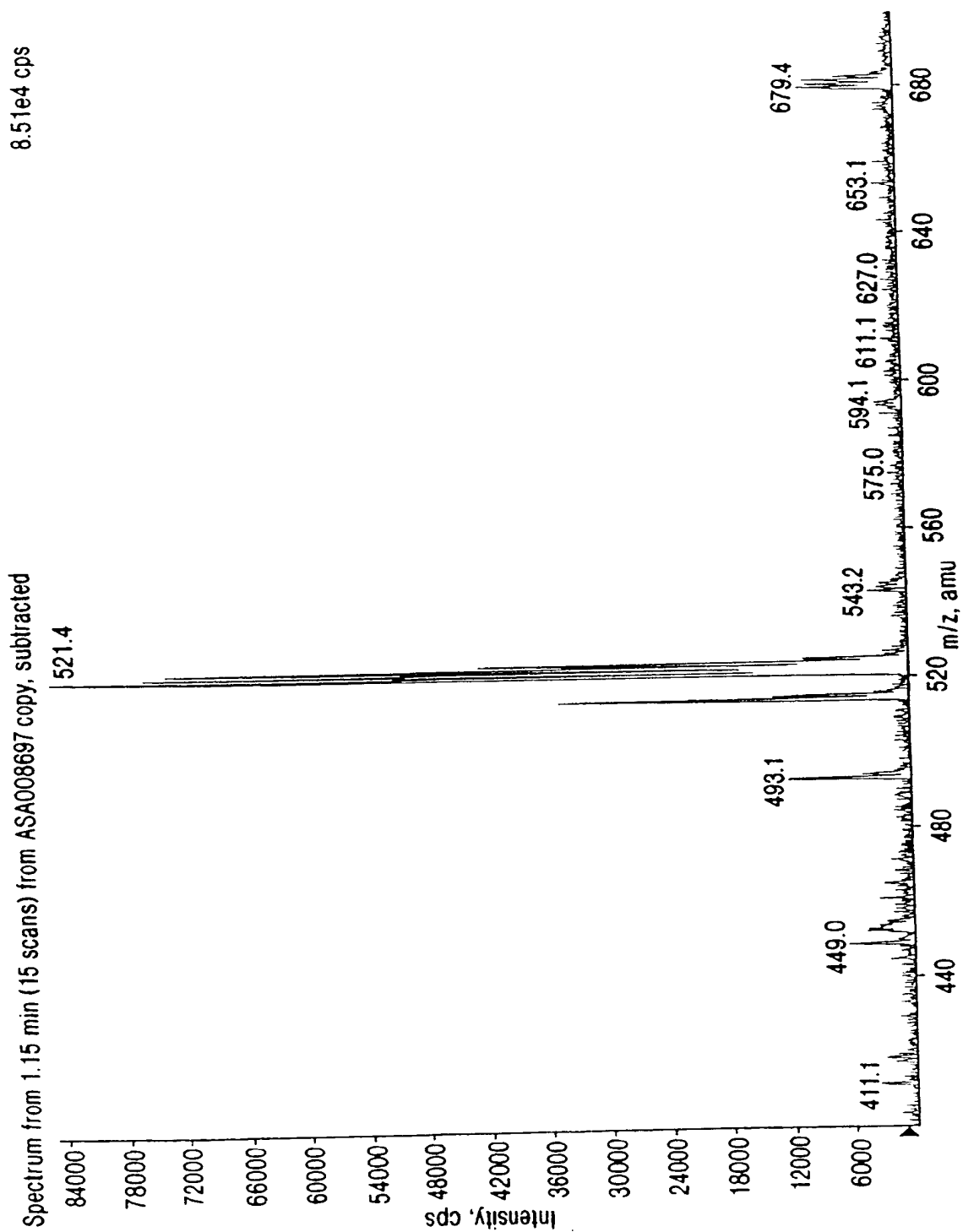


FIG. 147

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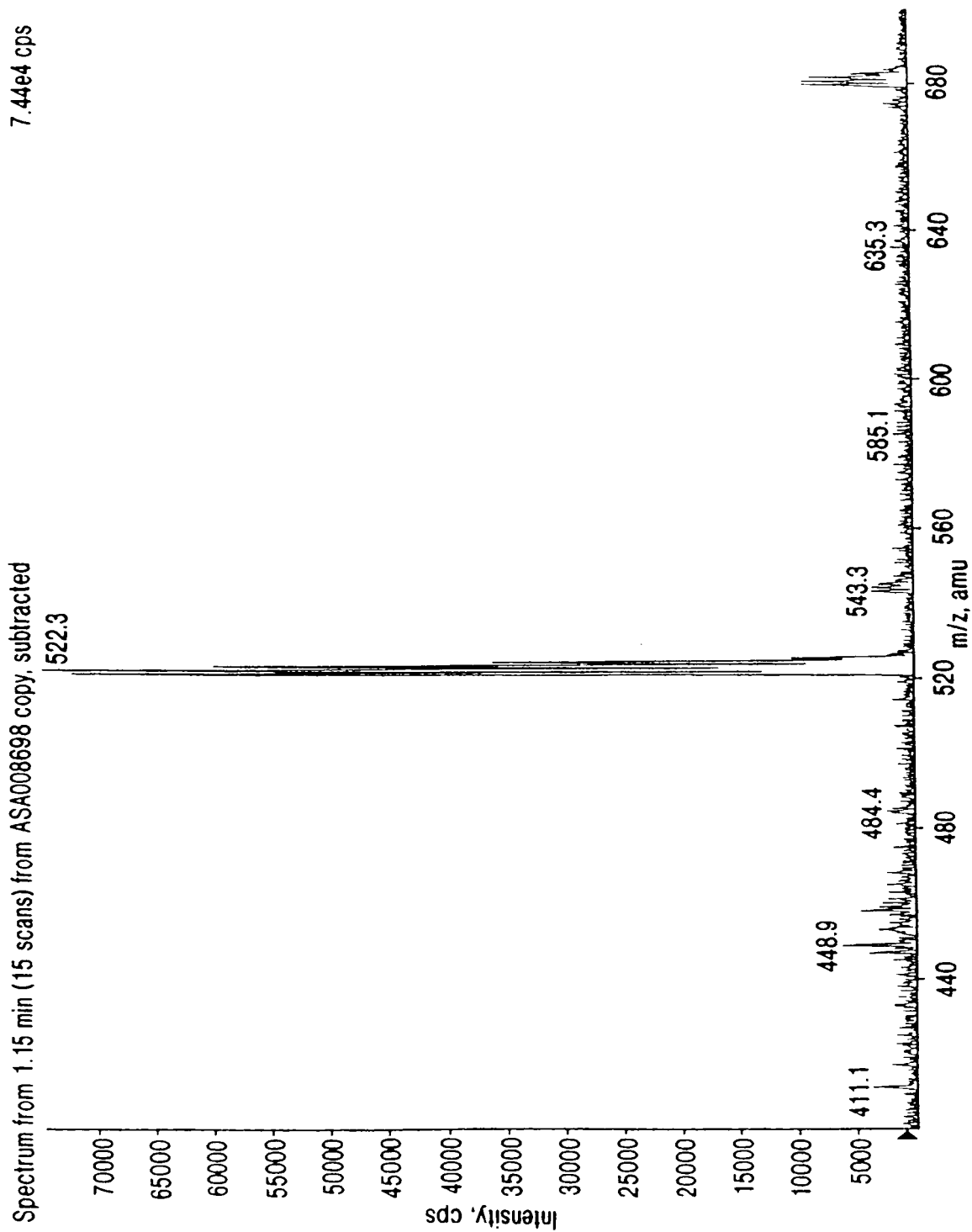


FIG. 148

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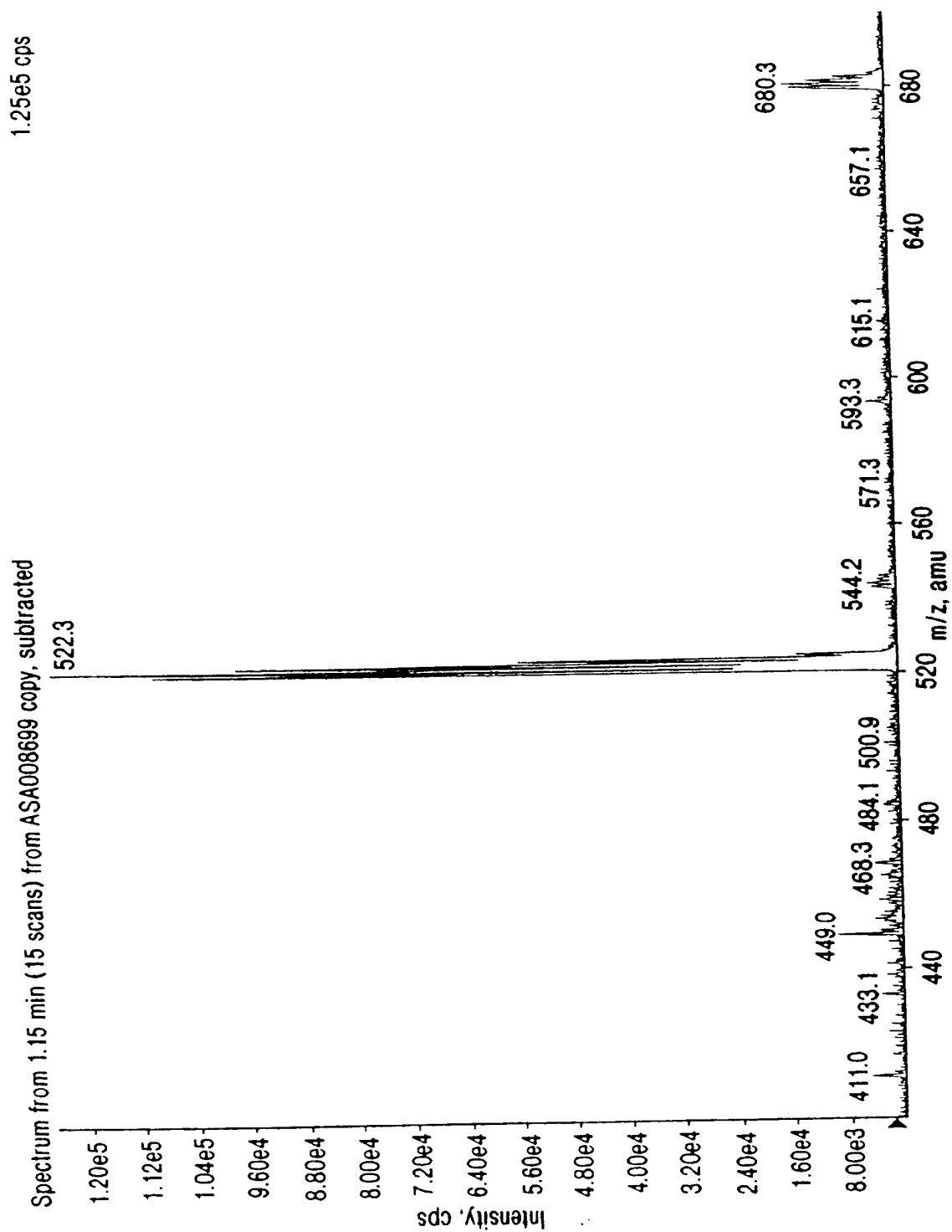


FIG. 149

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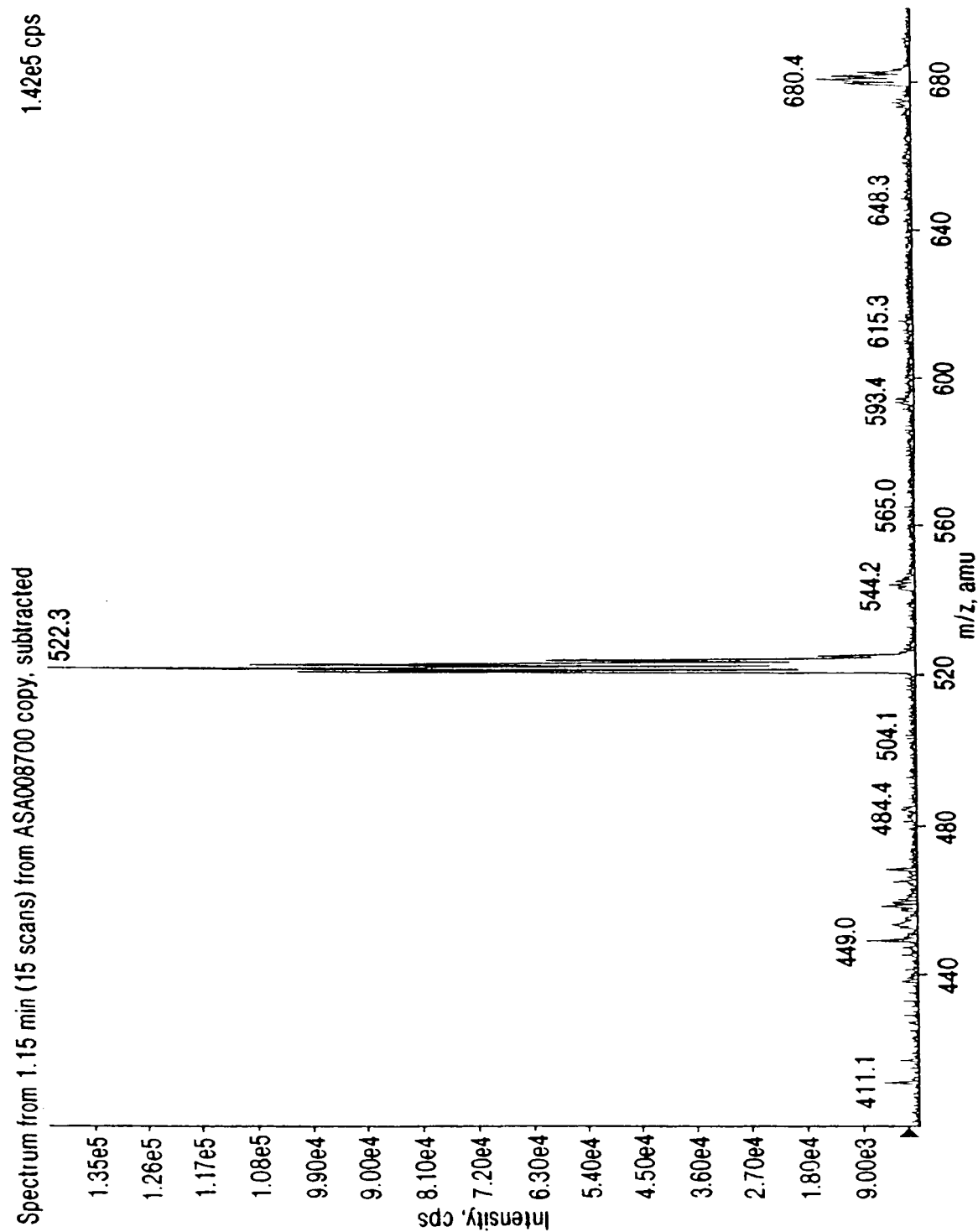


FIG. 150

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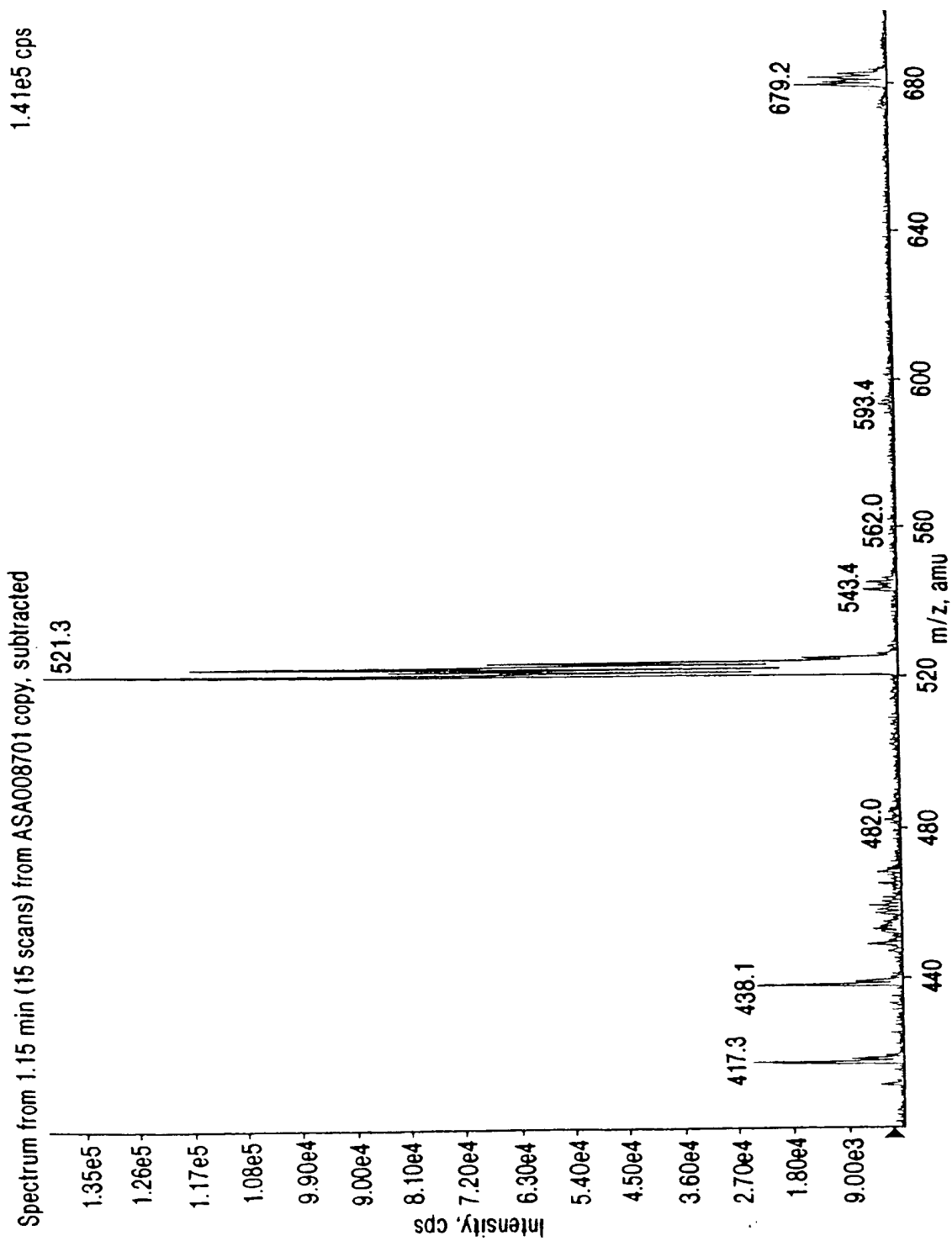


FIG. 151

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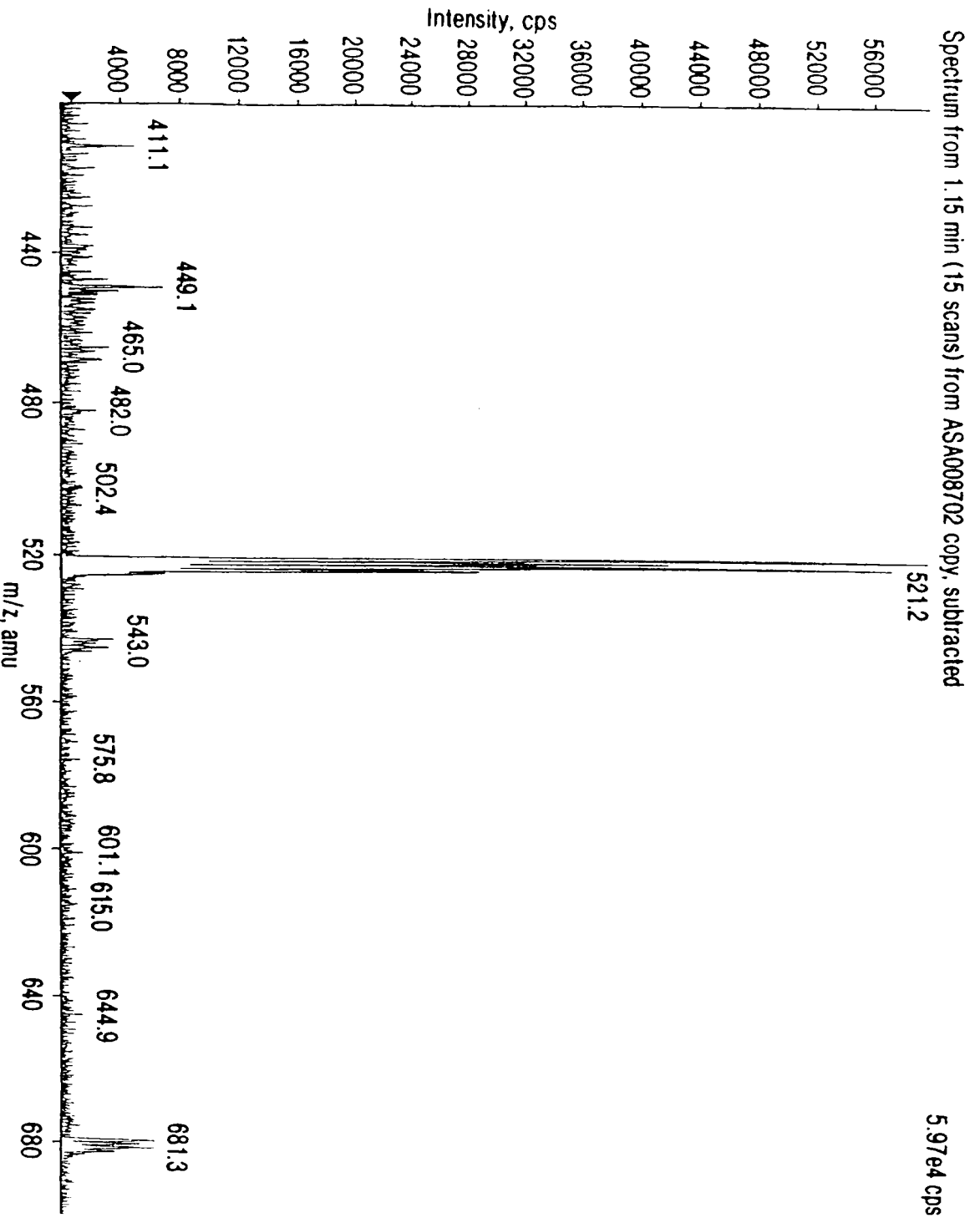


FIG. 152

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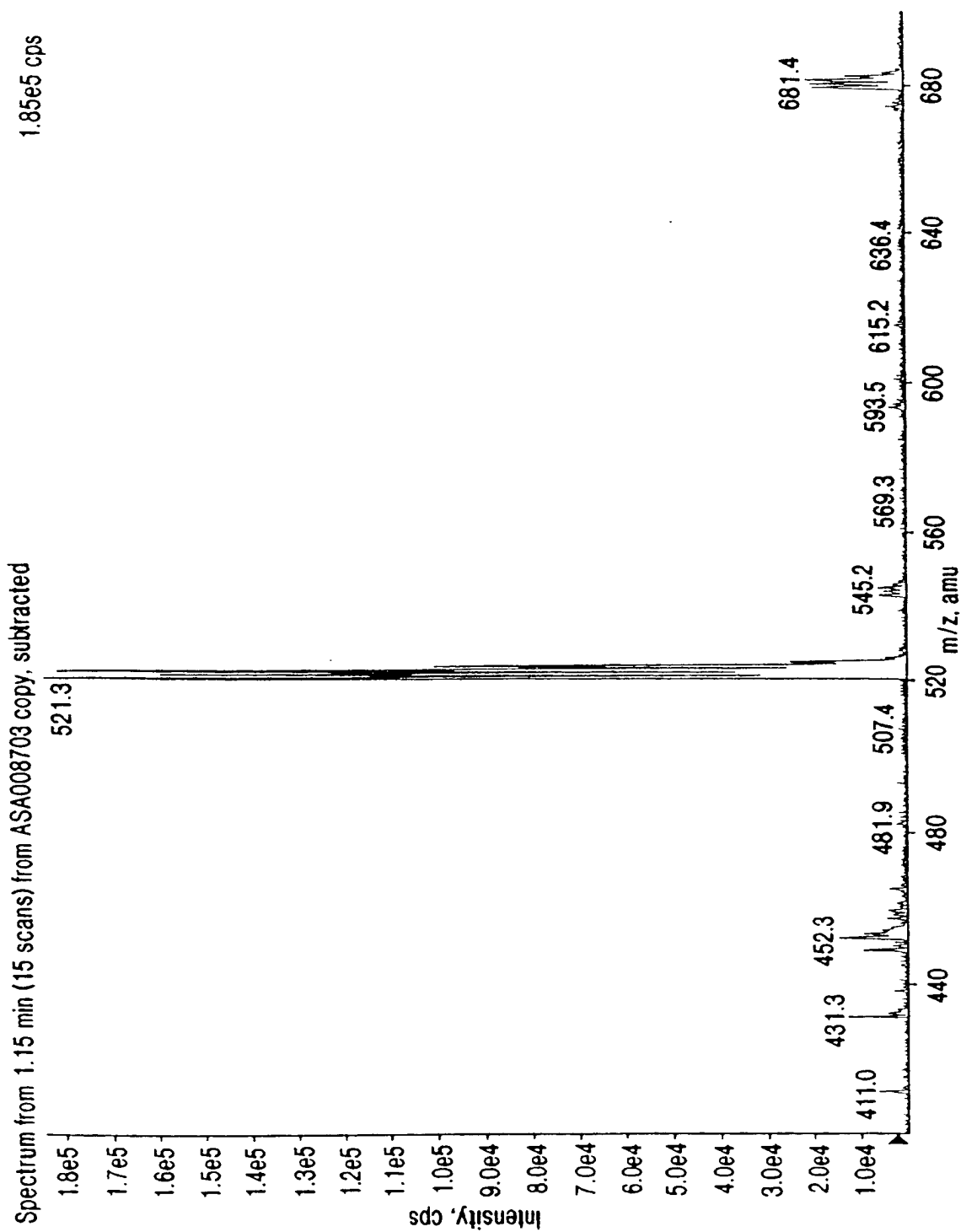


FIG. 153

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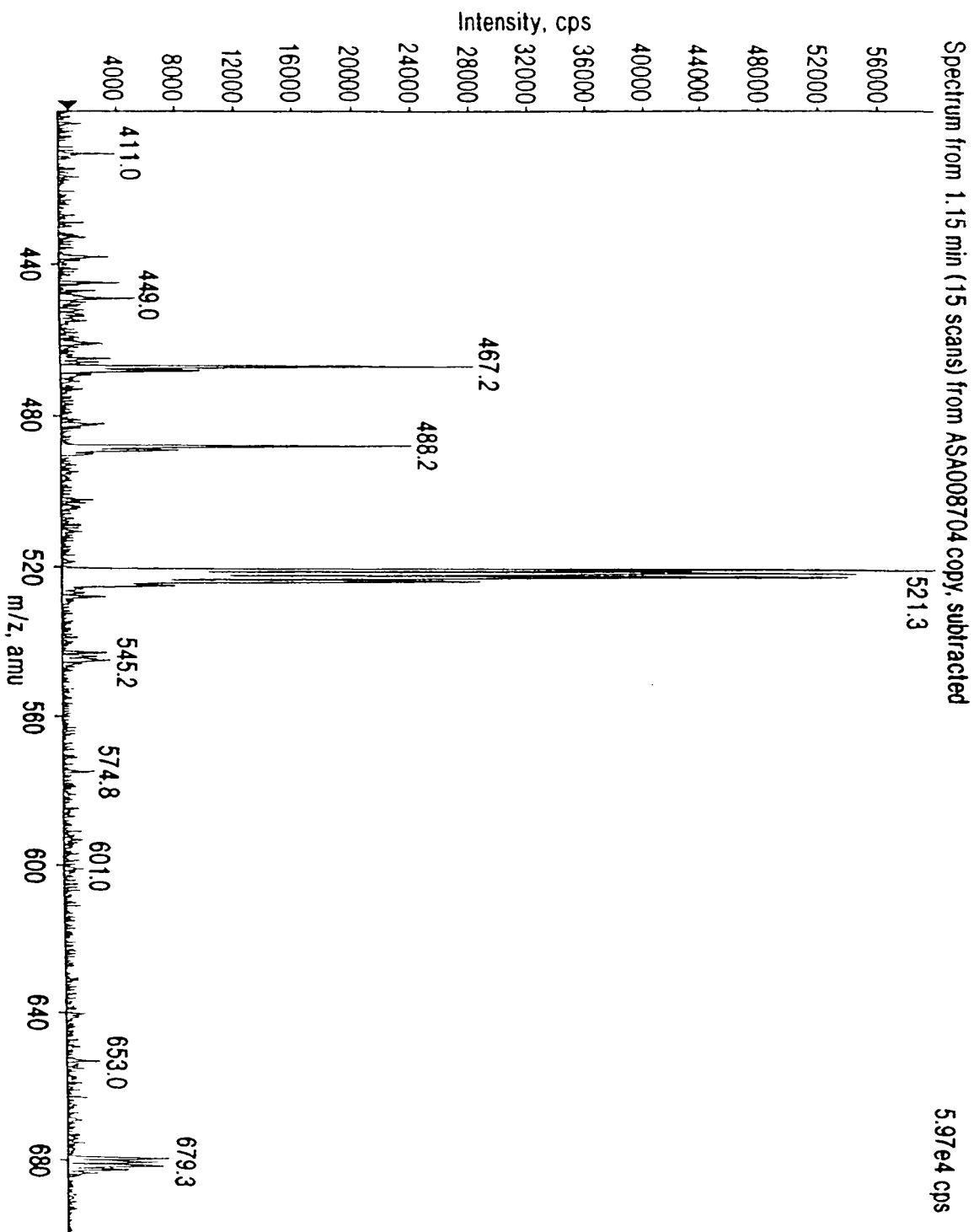


FIG. 154

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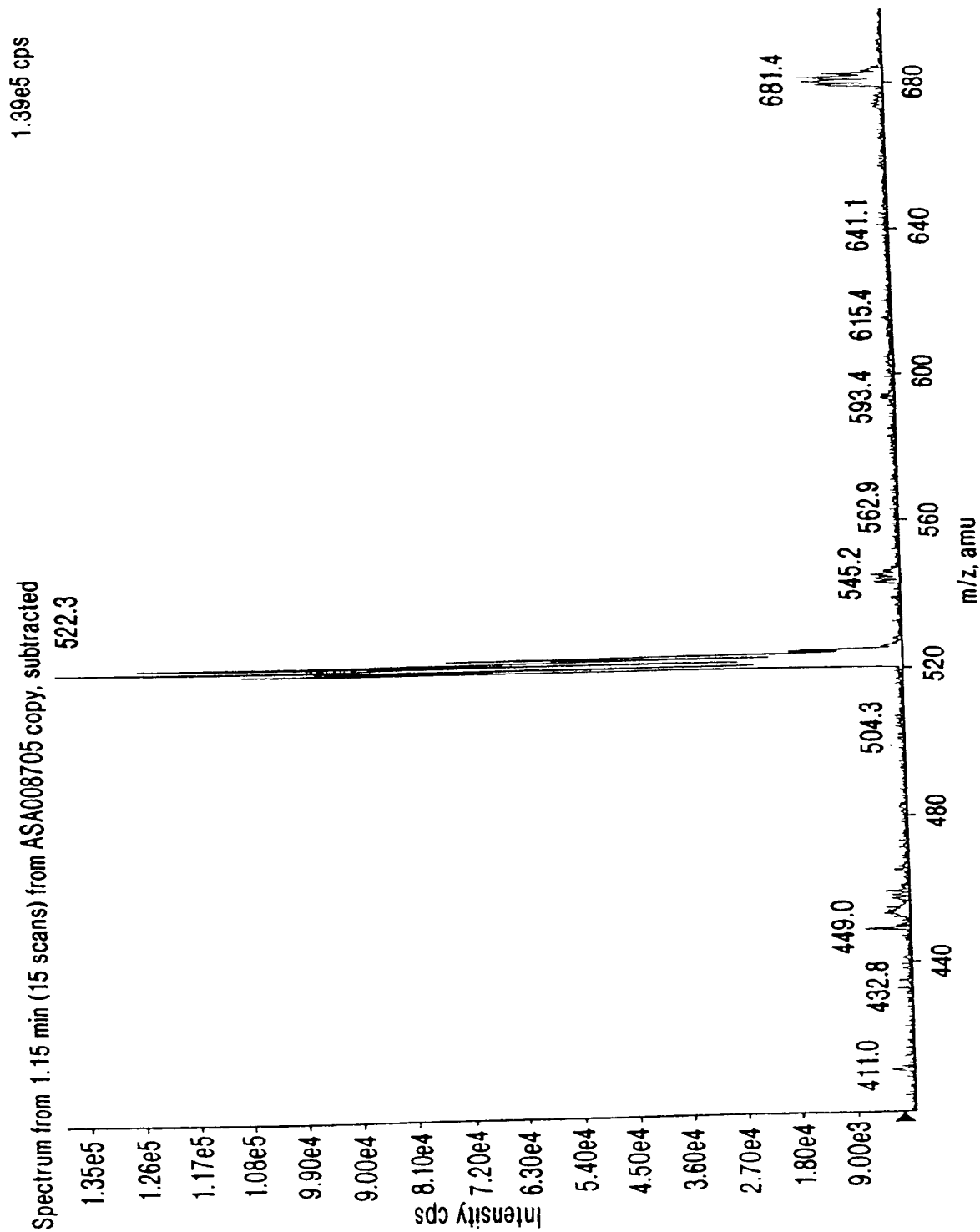


FIG. 155

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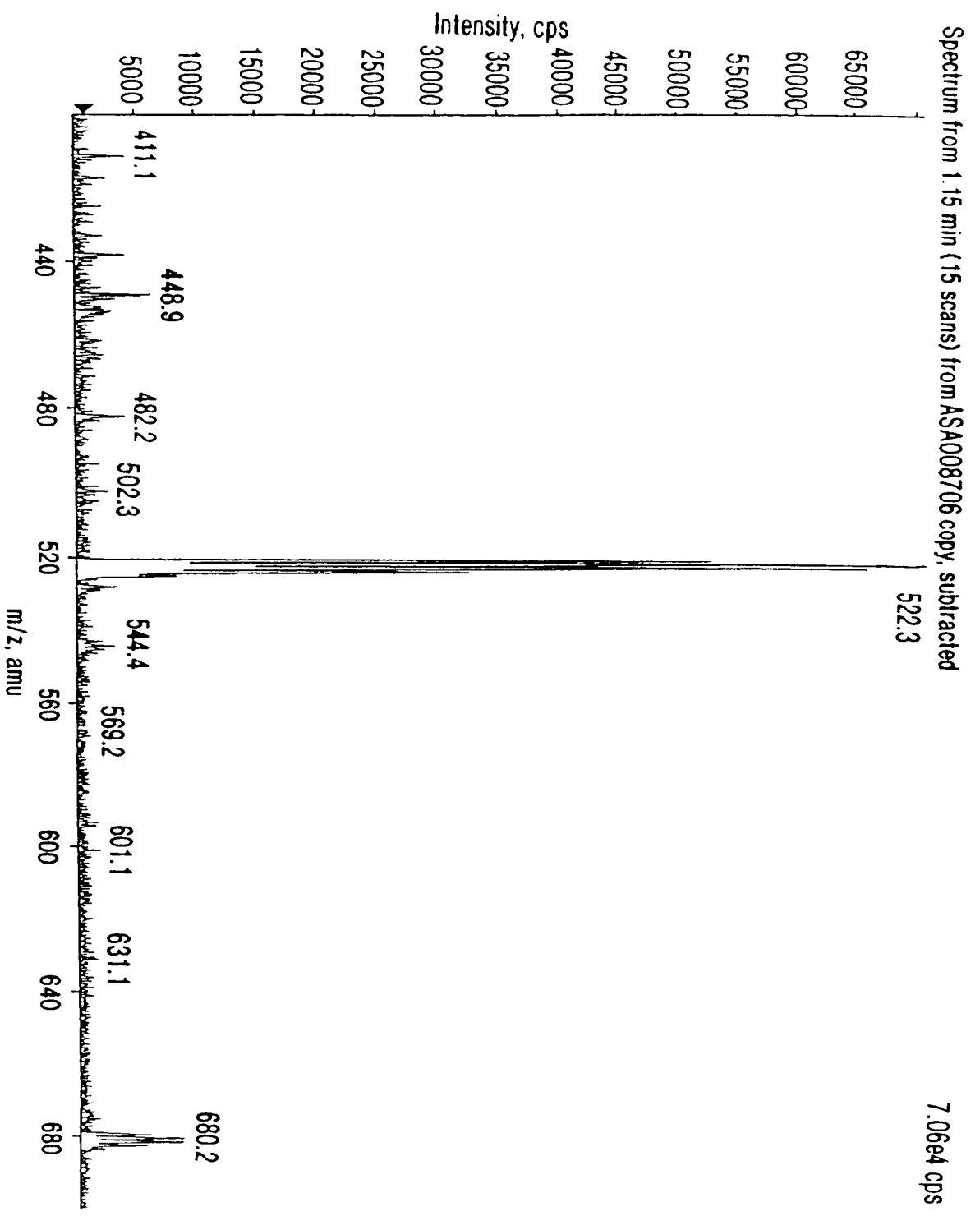


FIG. 156

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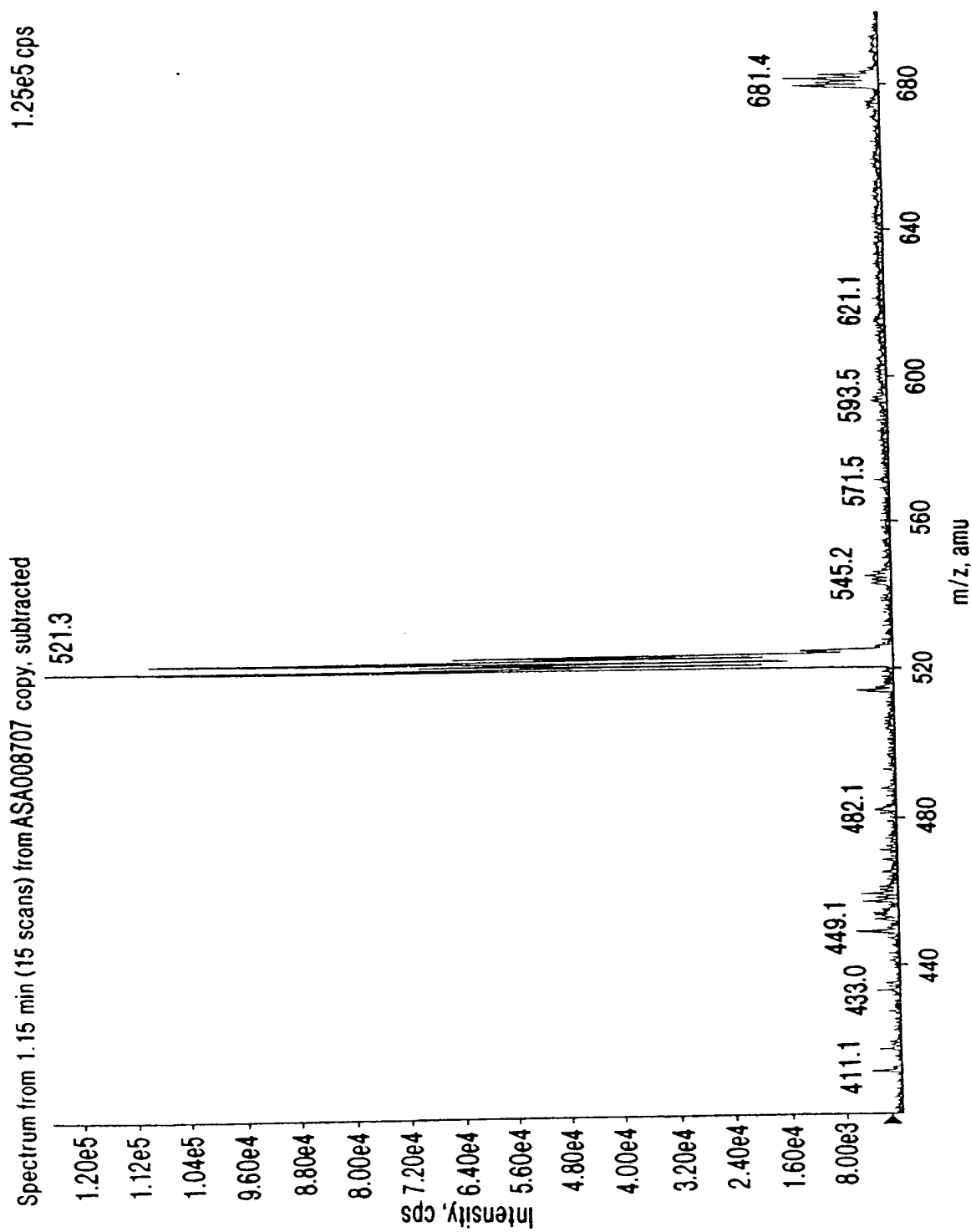


FIG. 157

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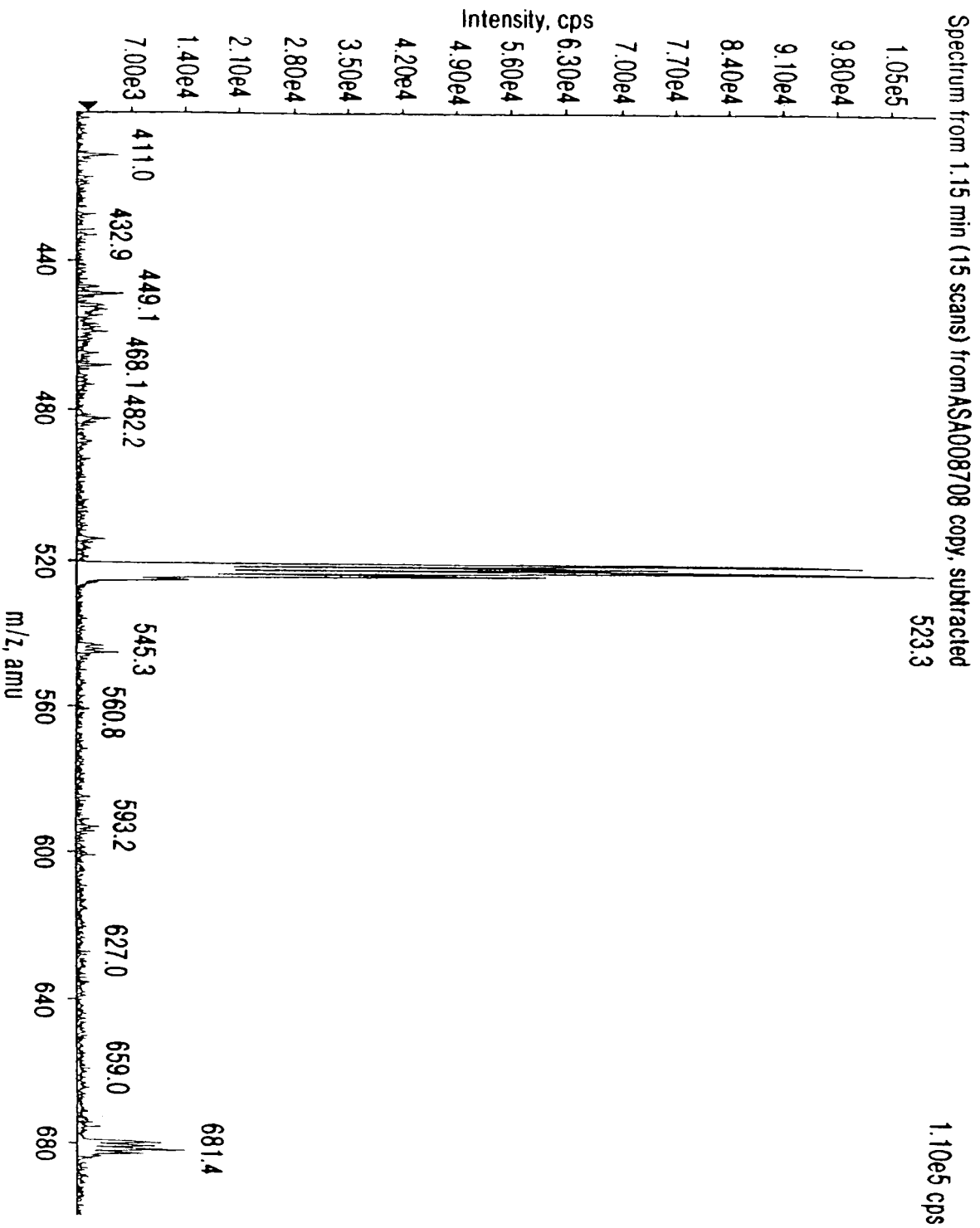


FIG. 158

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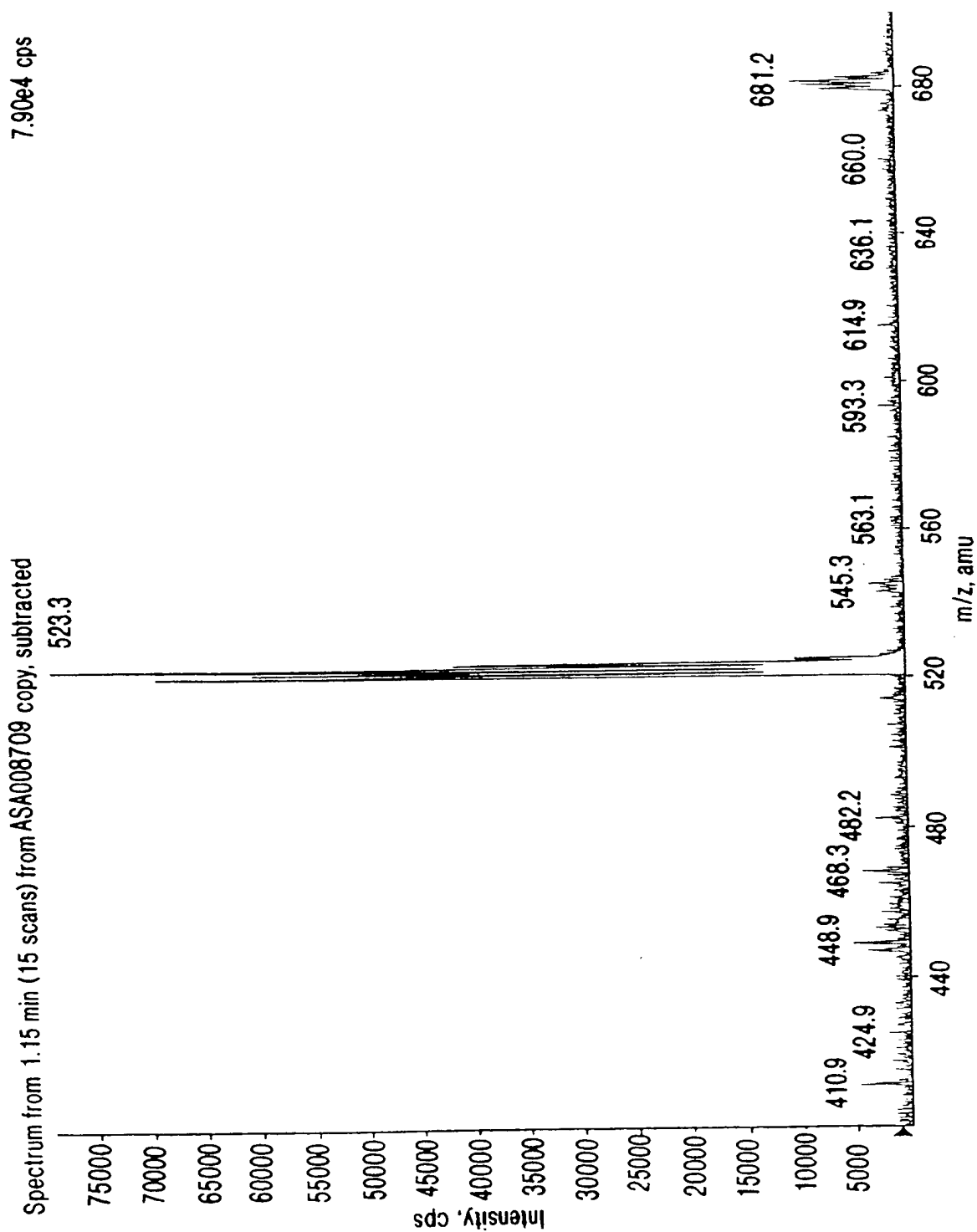


FIG. 159

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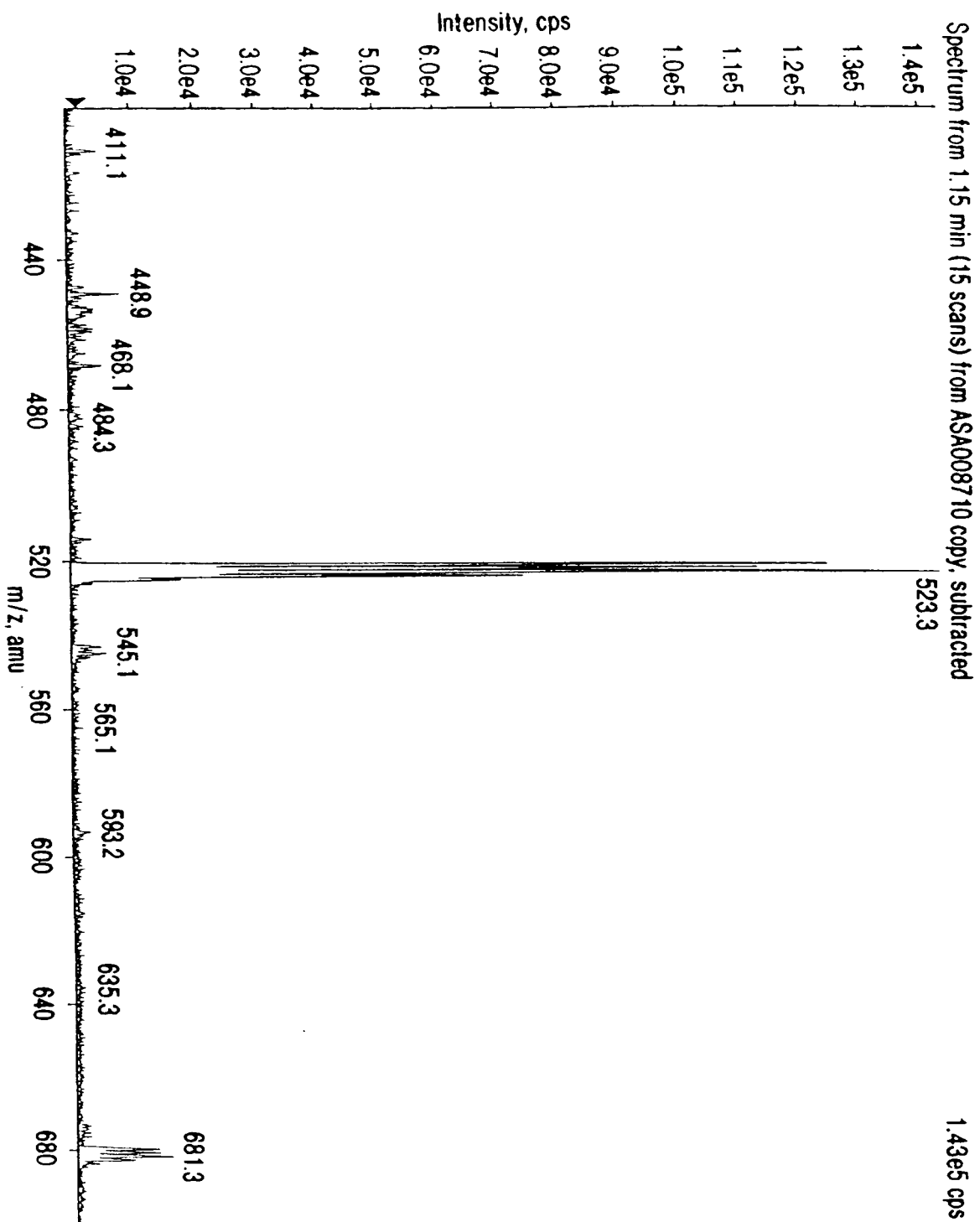


FIG. 160

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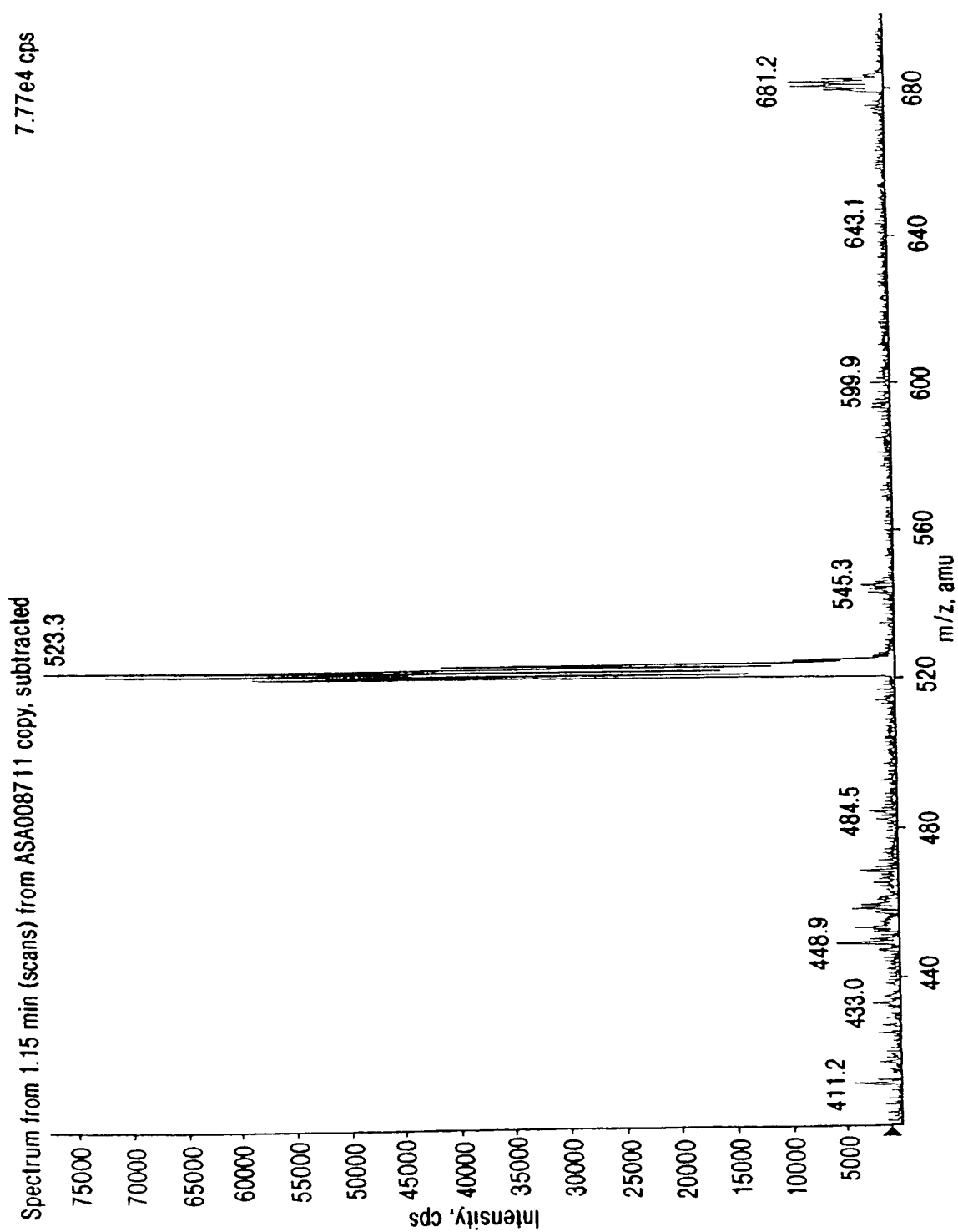


FIG. 161

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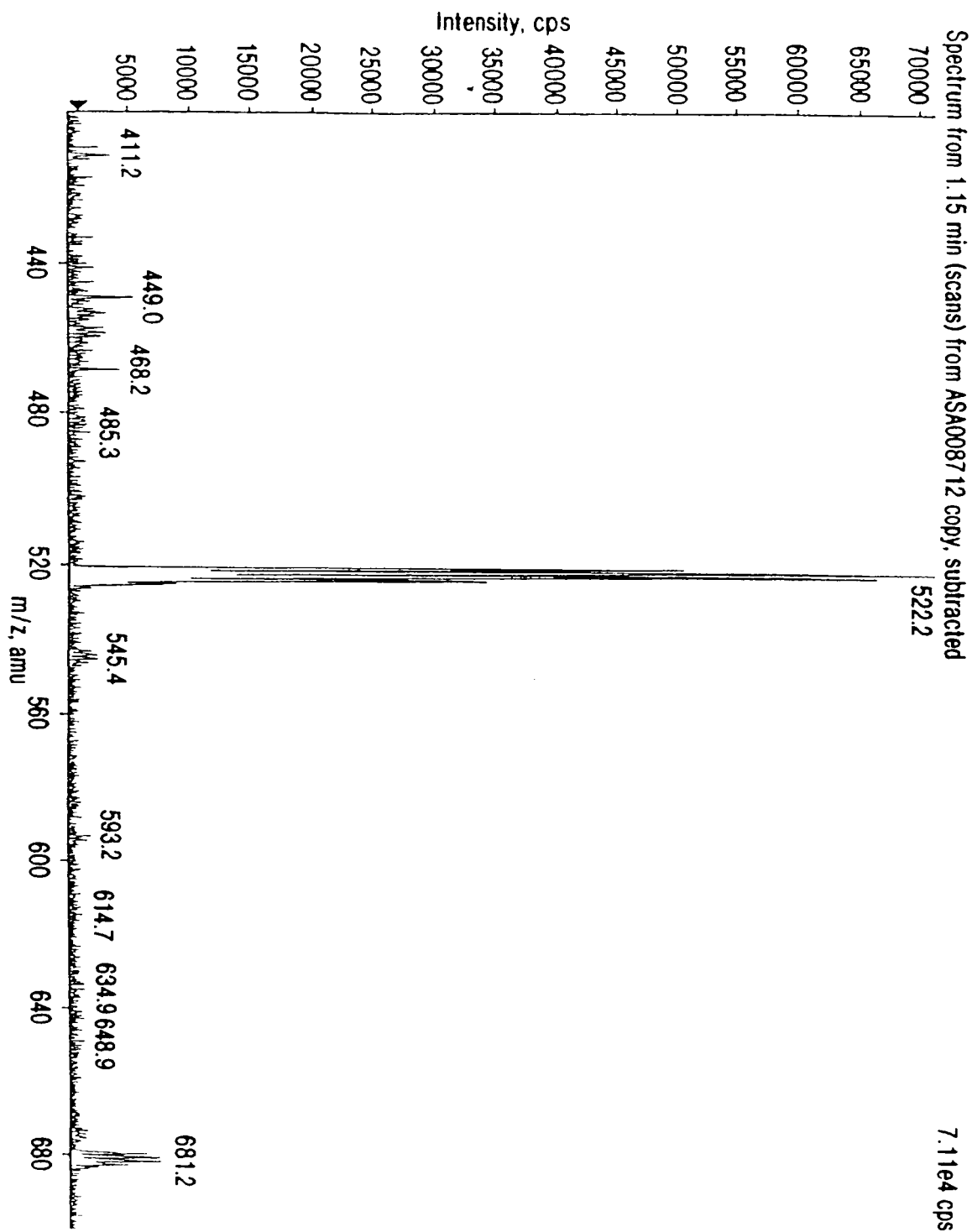


FIG. 162

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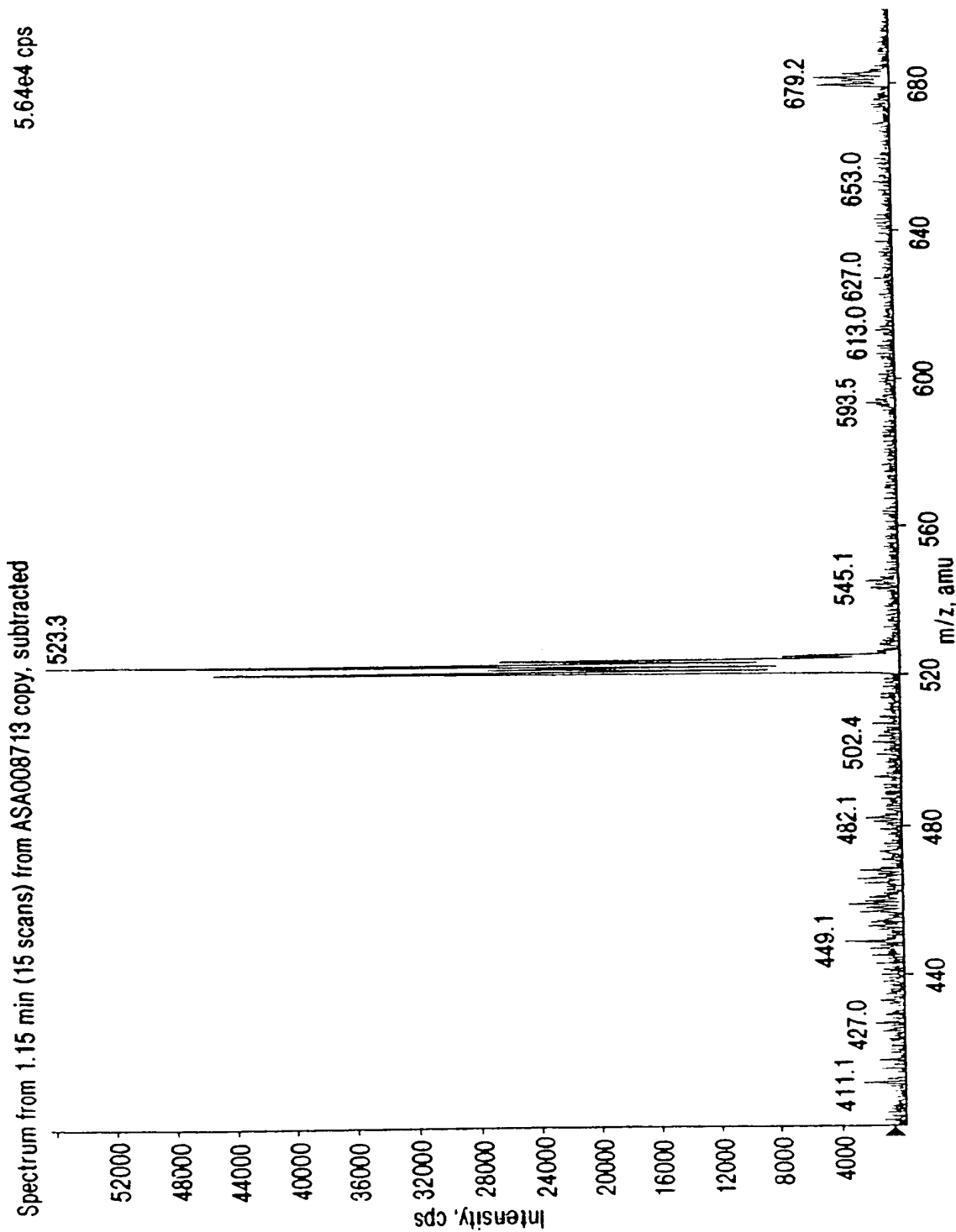


FIG. 163

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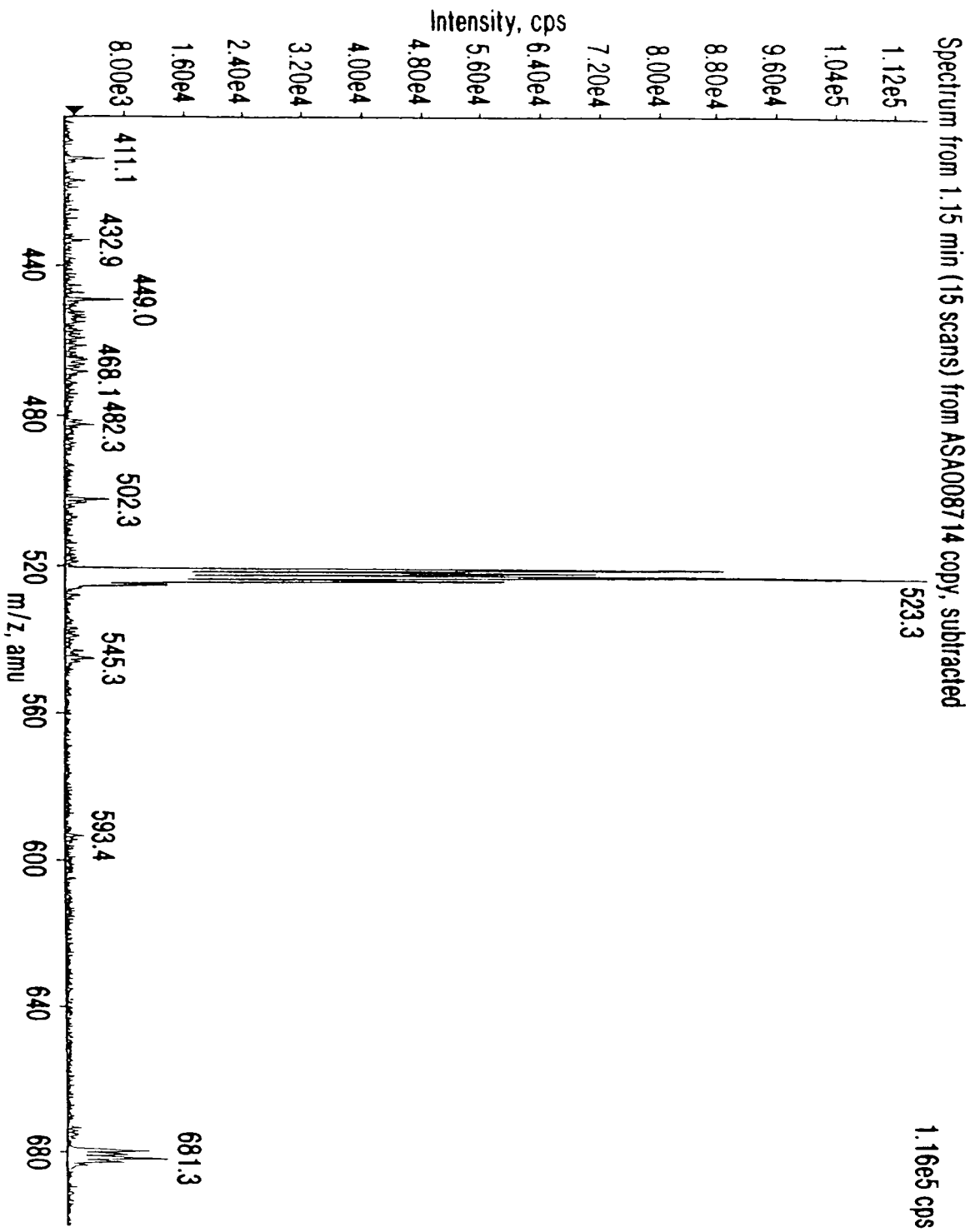


FIG. 164

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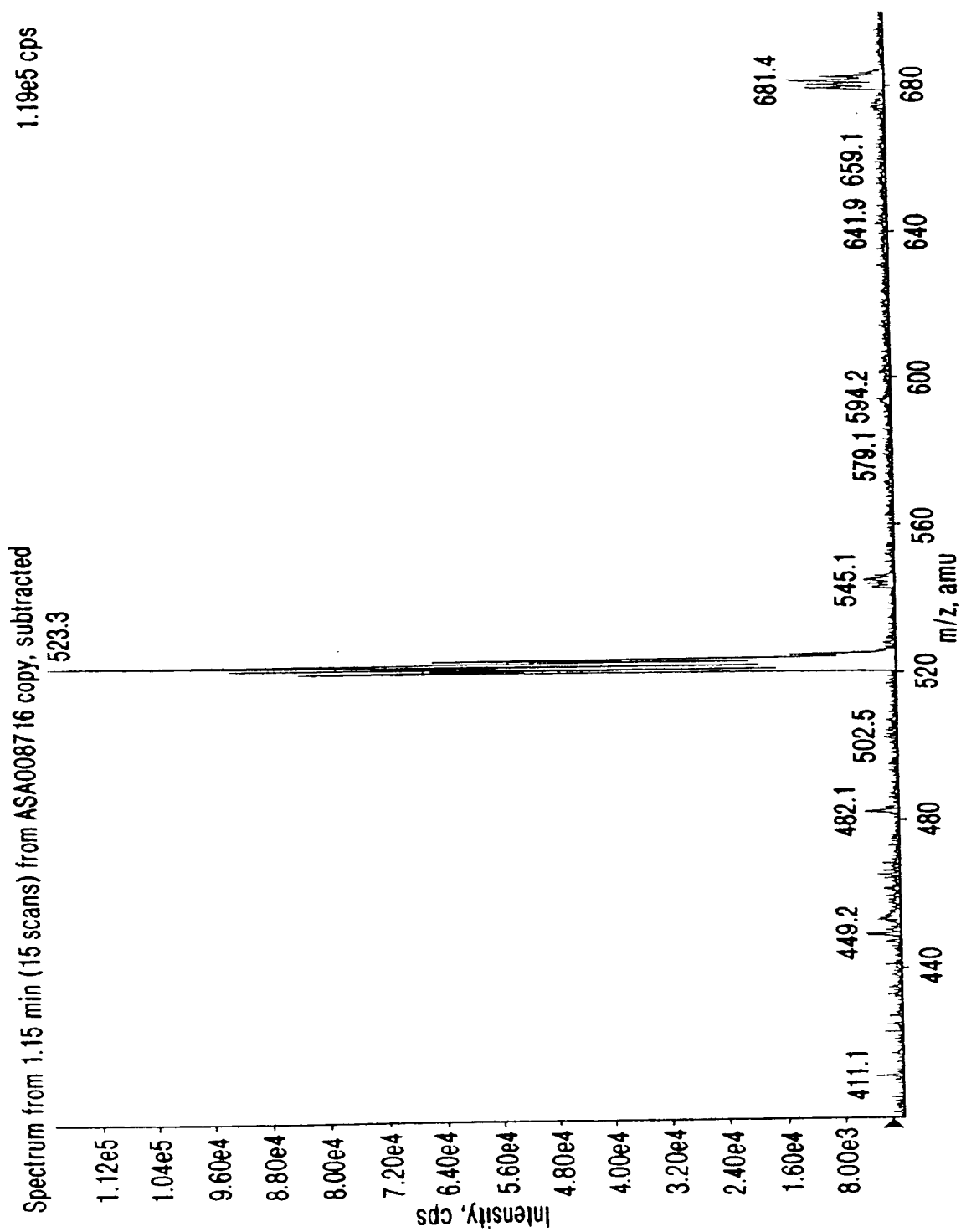


FIG. 165

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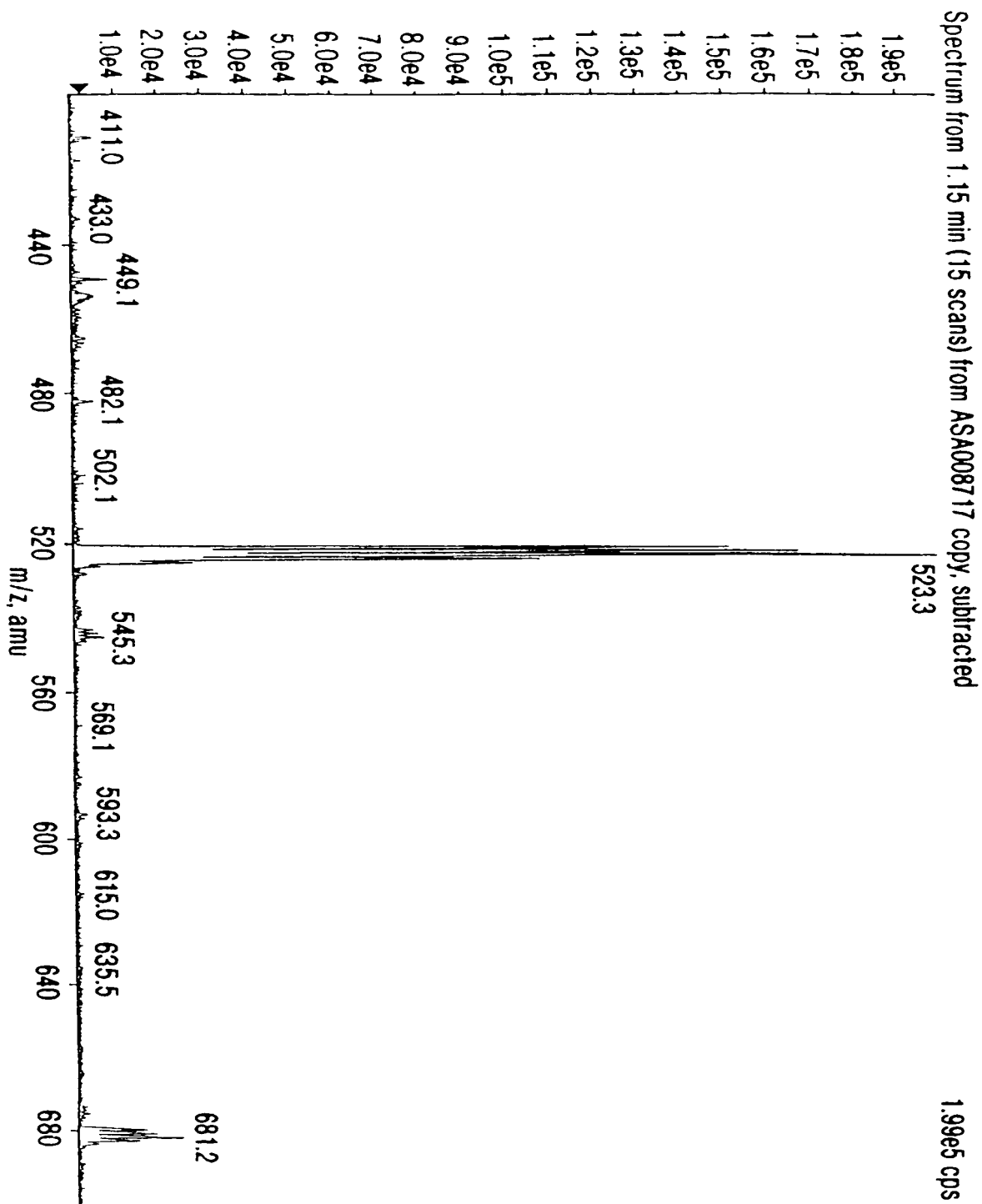


FIG. 166

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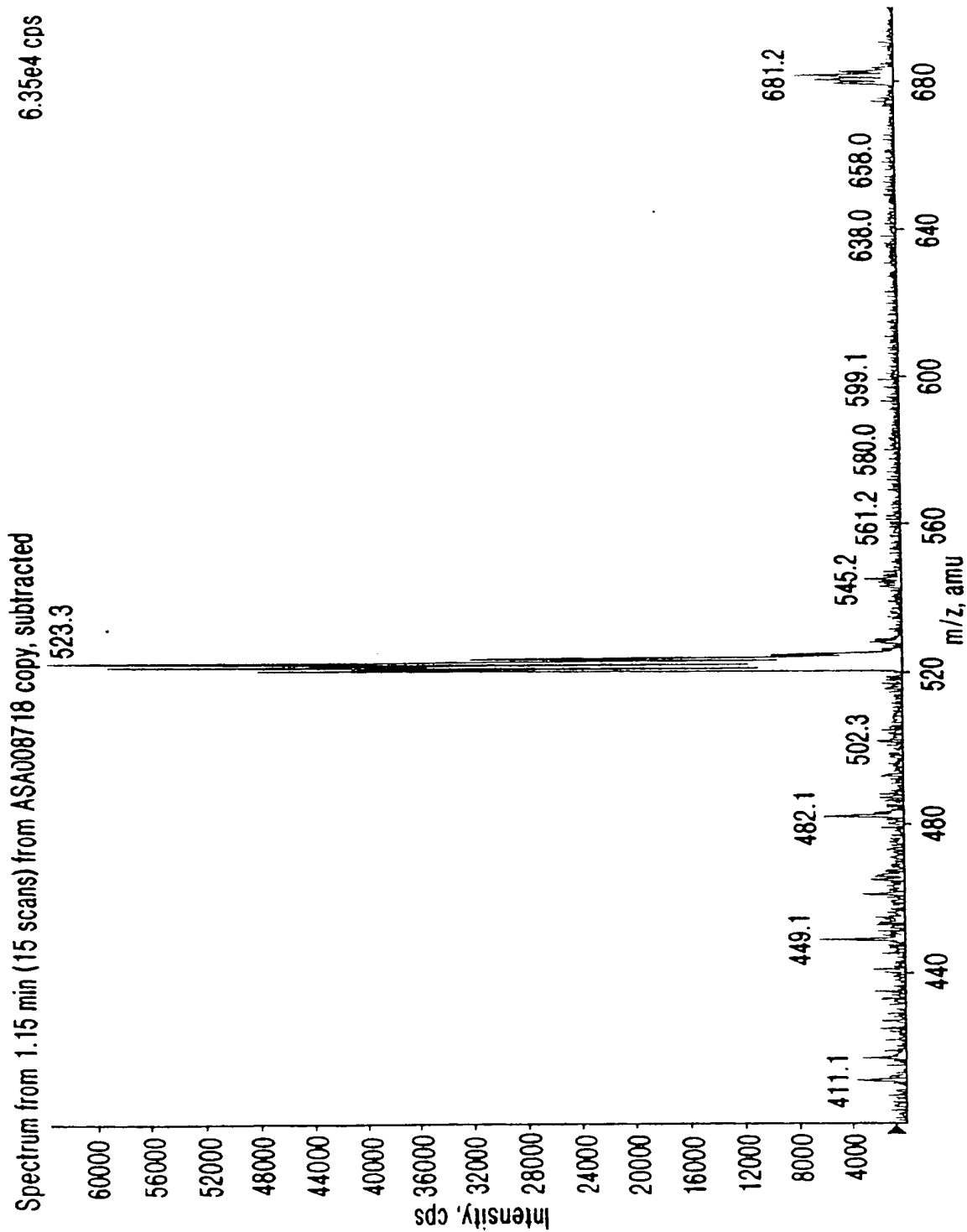


FIG. 167

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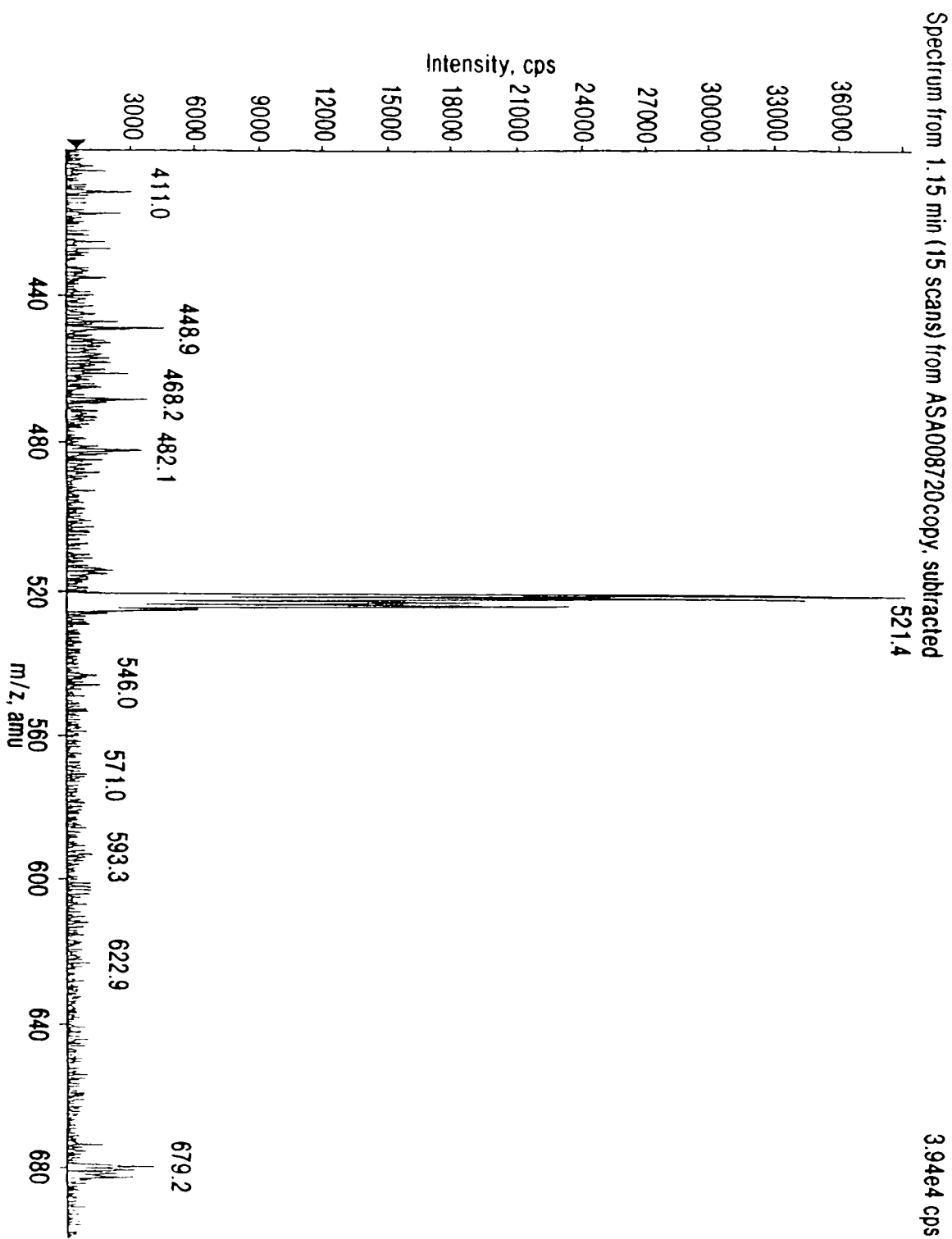


FIG. 168

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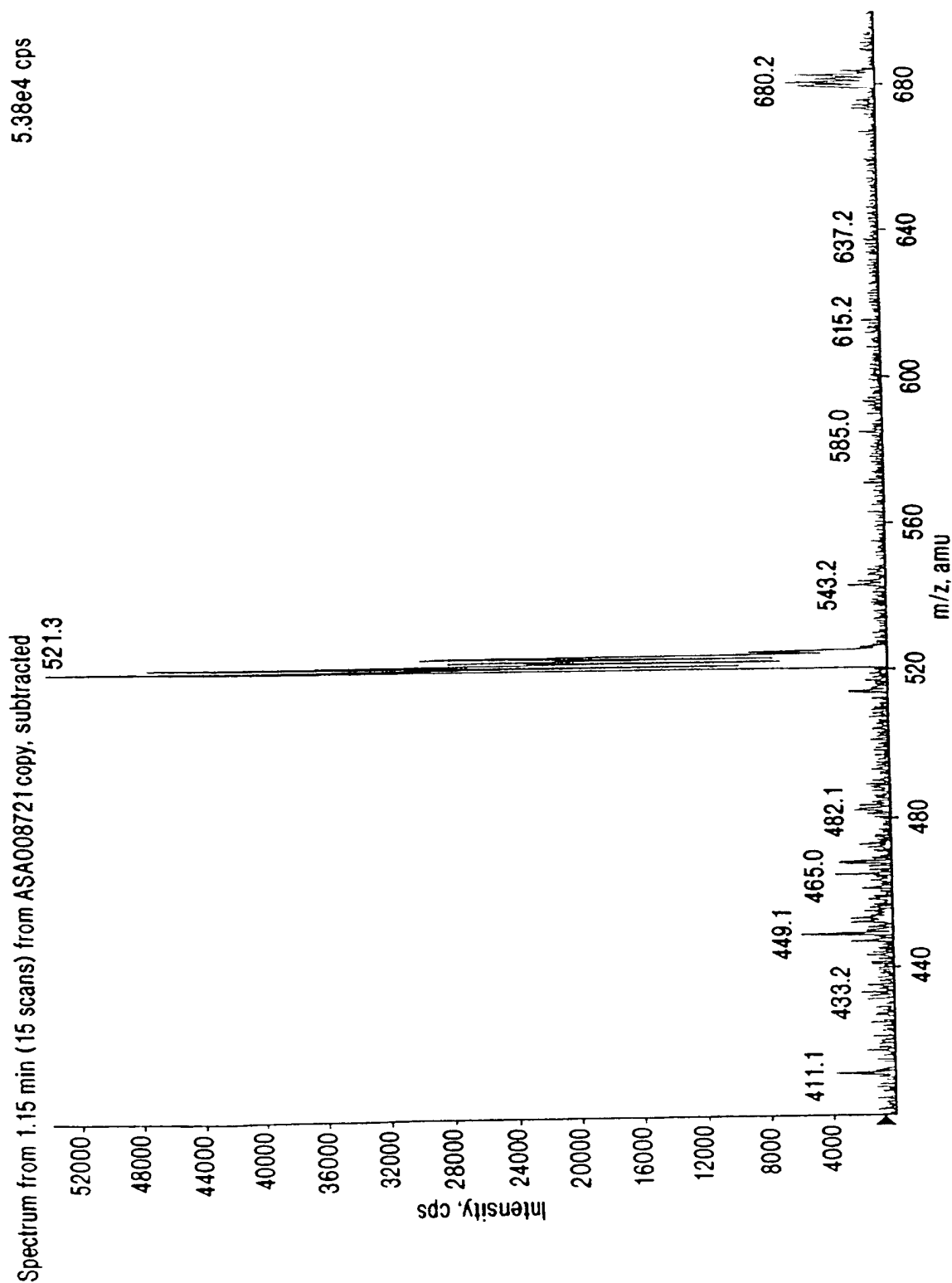


FIG. 169

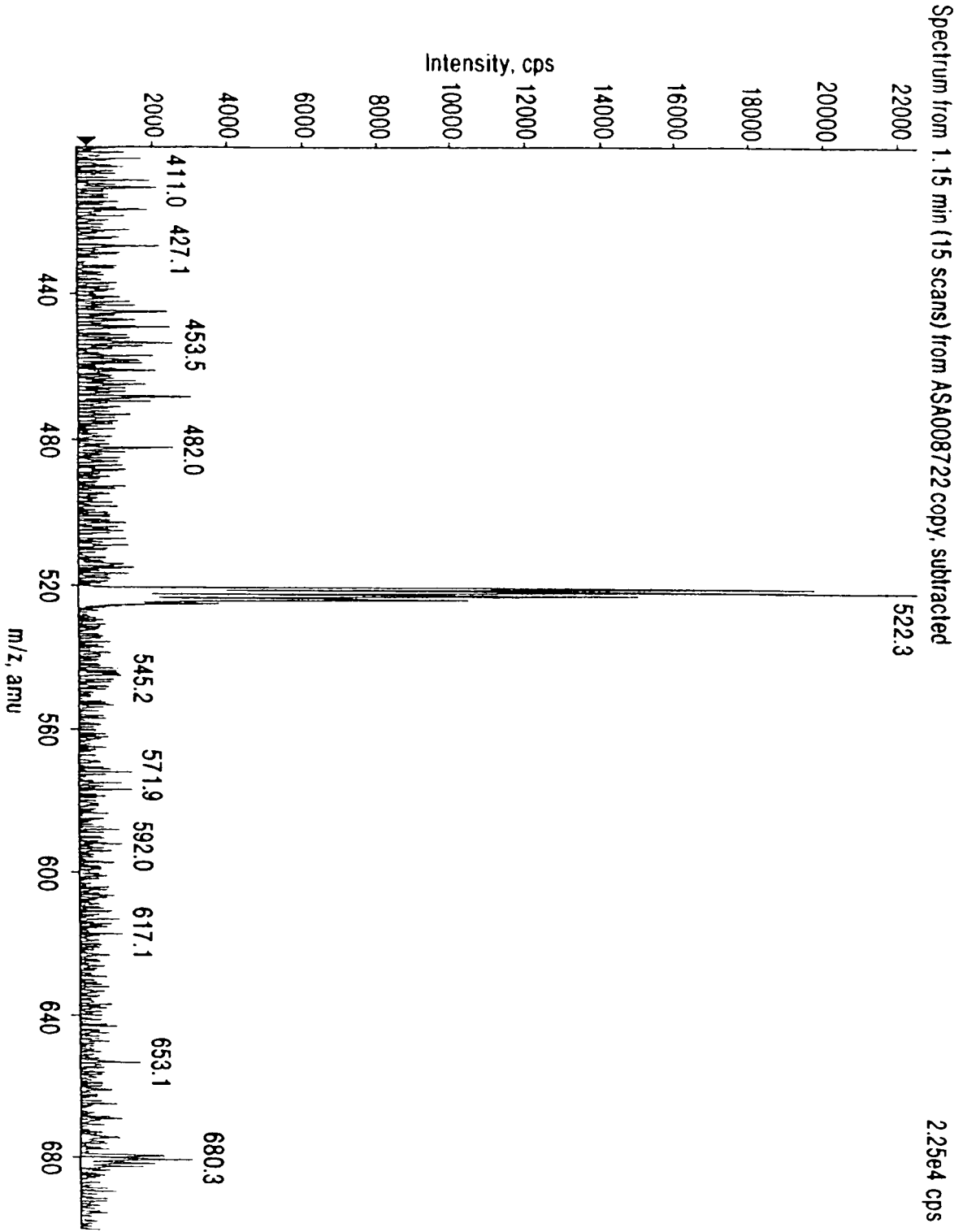


FIG. 170

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5.77e4 cps

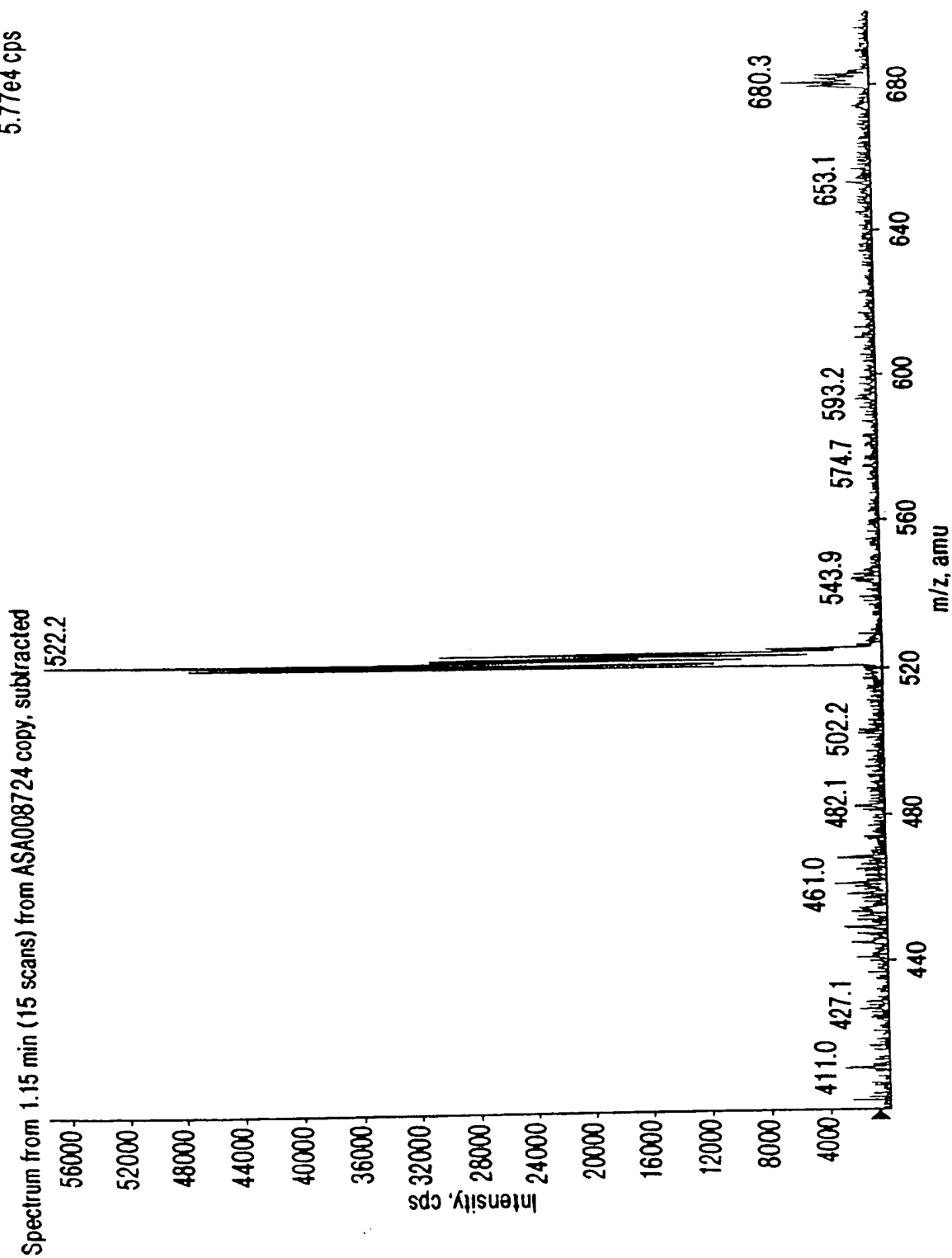


FIG. 171

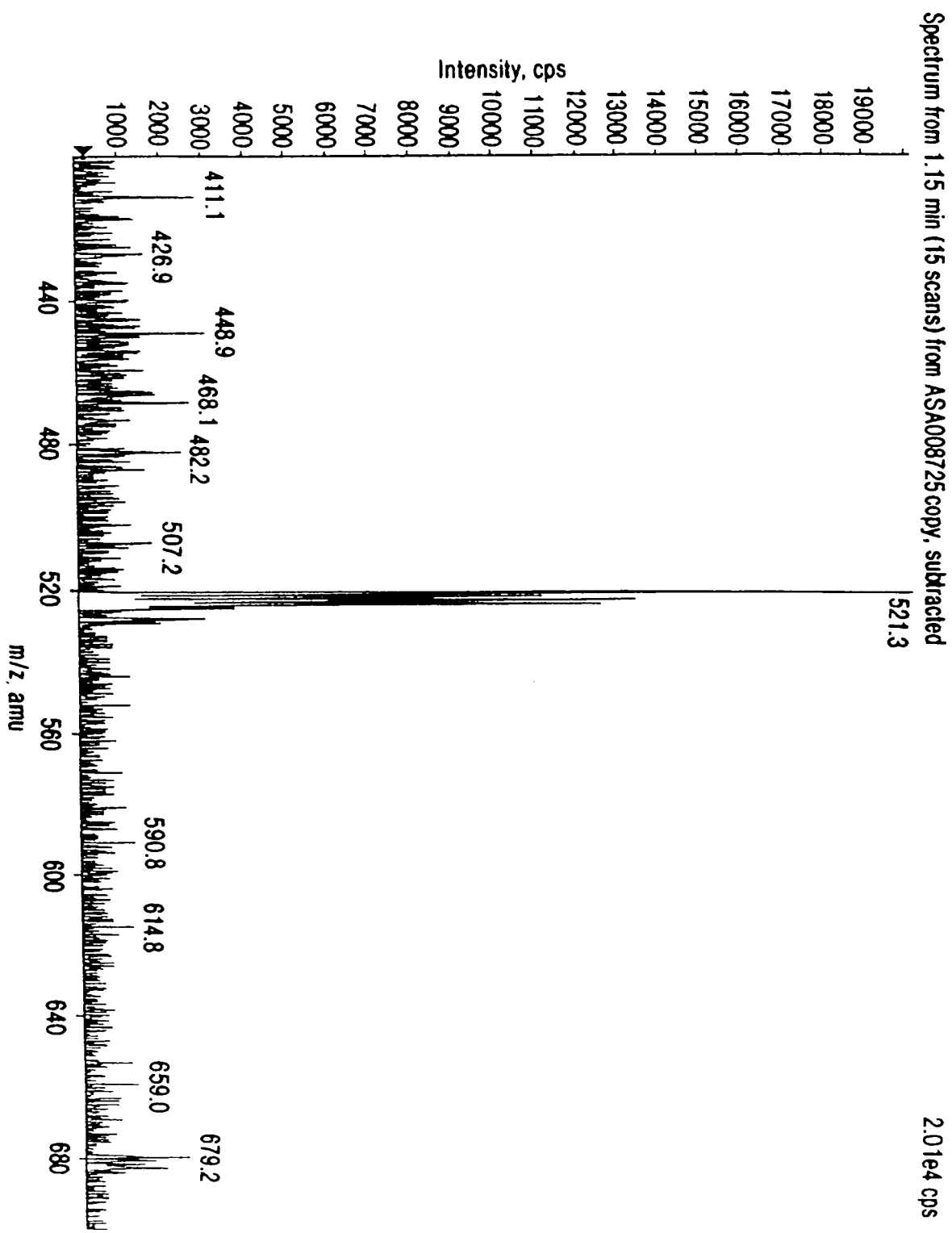


FIG. 172

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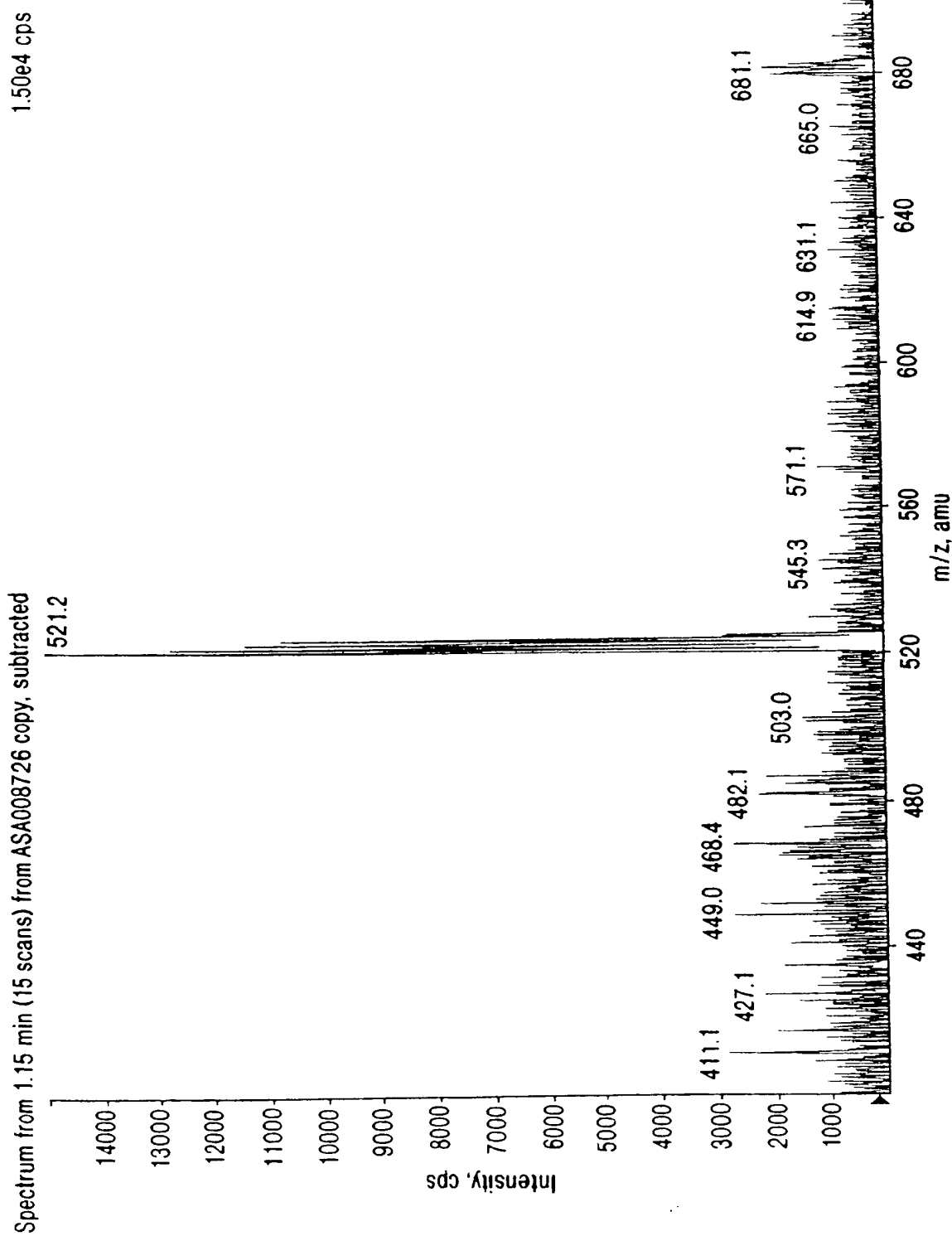


FIG. 173

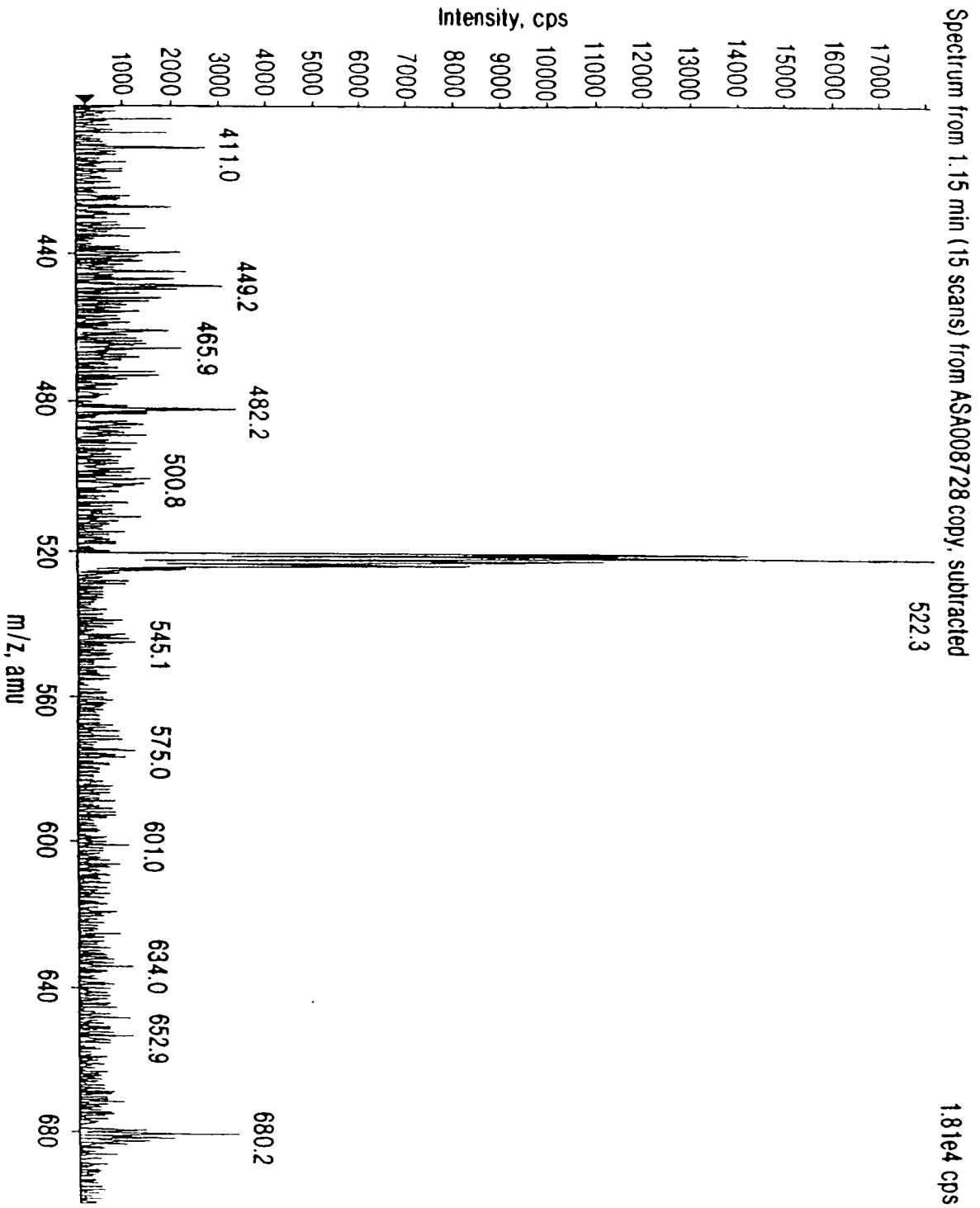


FIG. 174

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2.17e4 cps

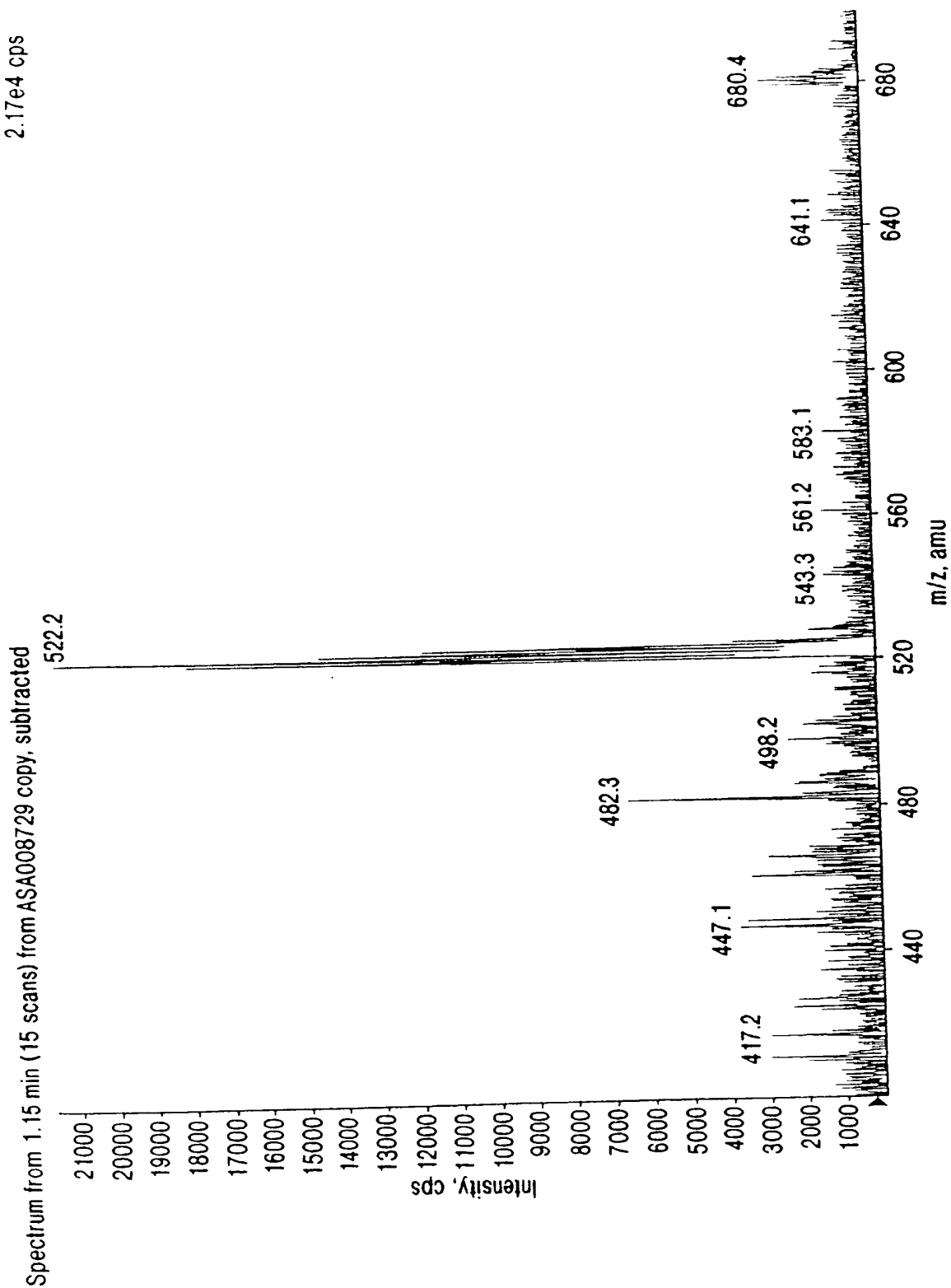


FIG. 175

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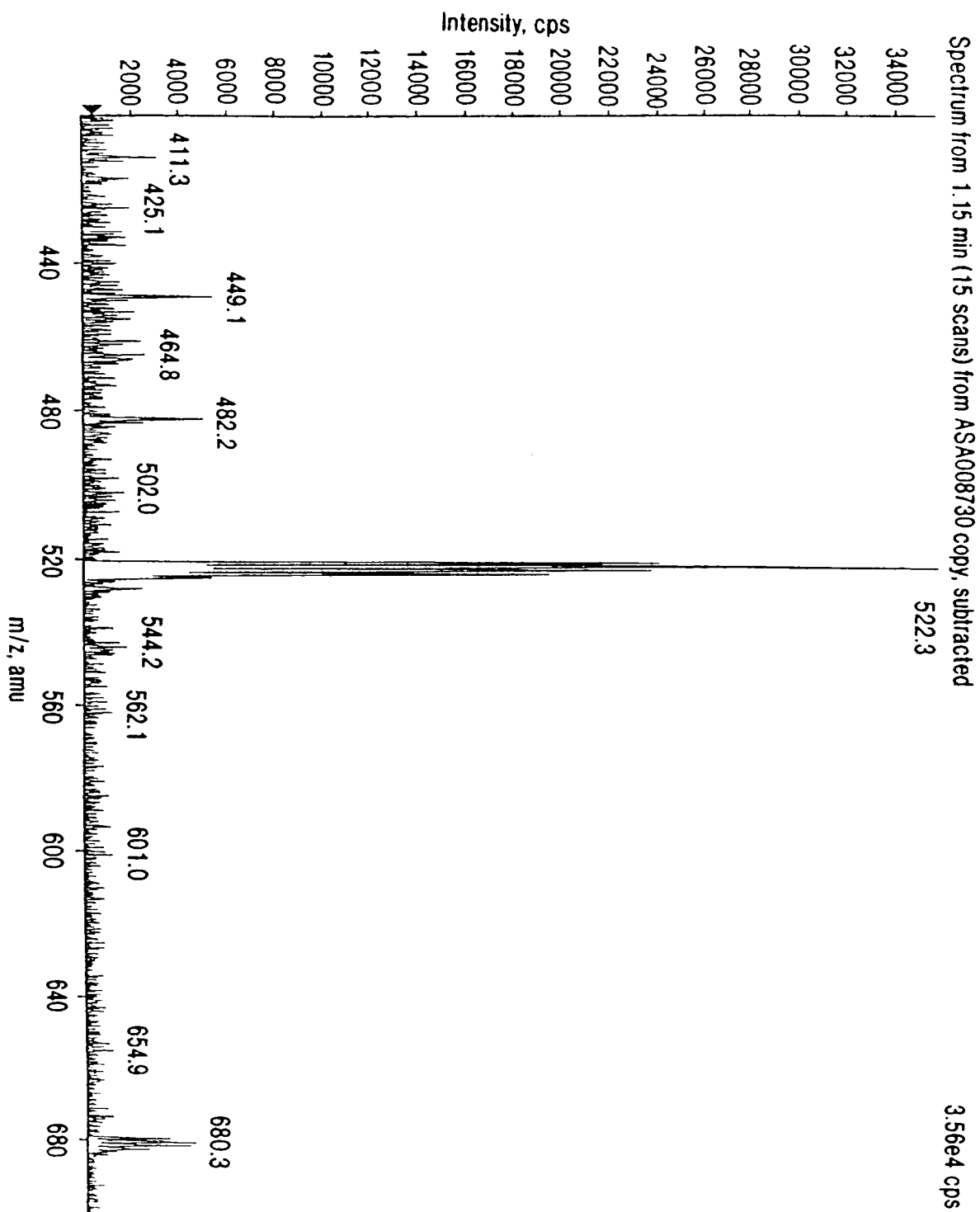


FIG. 176

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1.07e5 cps

Spectrum from 1.15 min (15 scans) from ASA008731 copy, subtracted

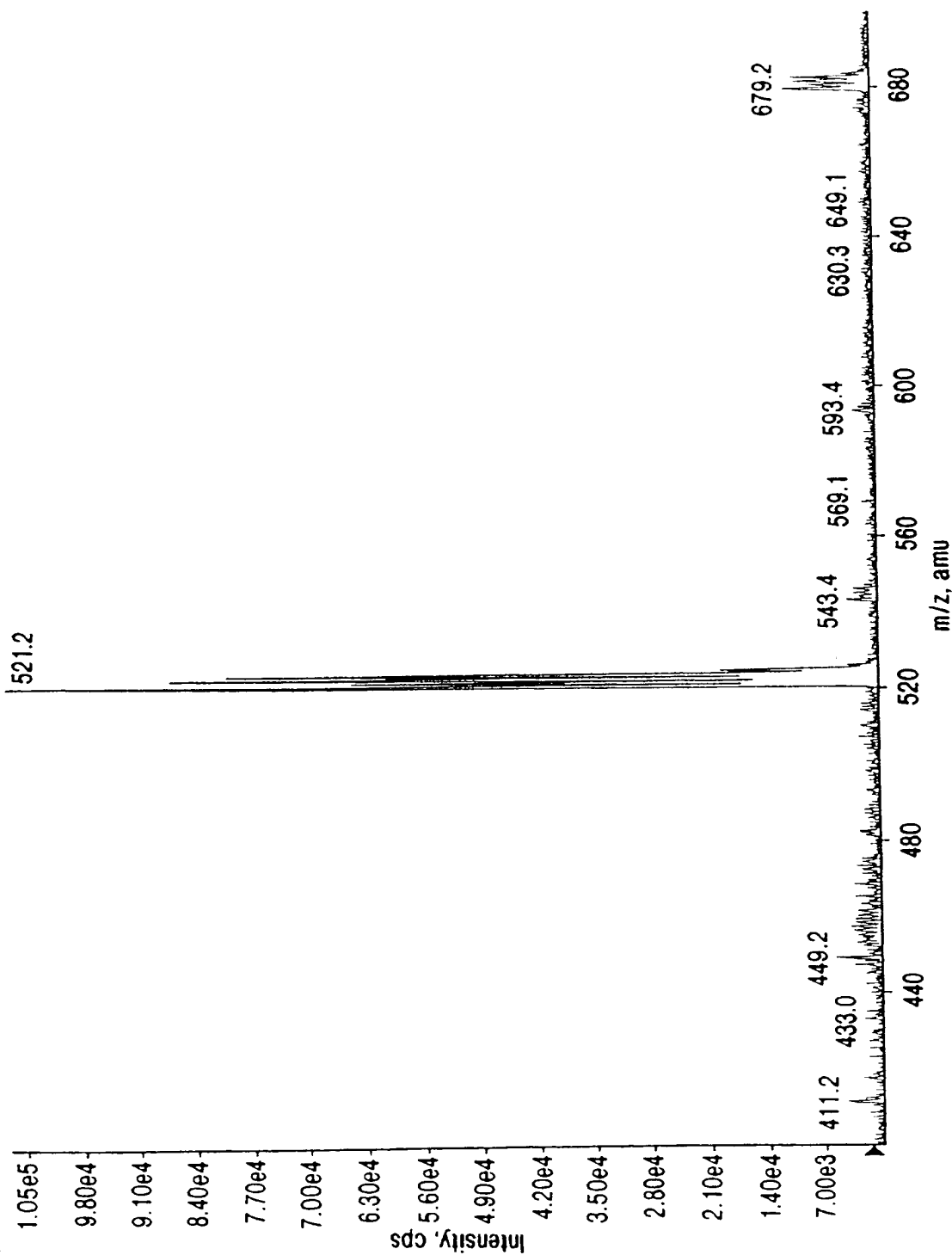


FIG. 177

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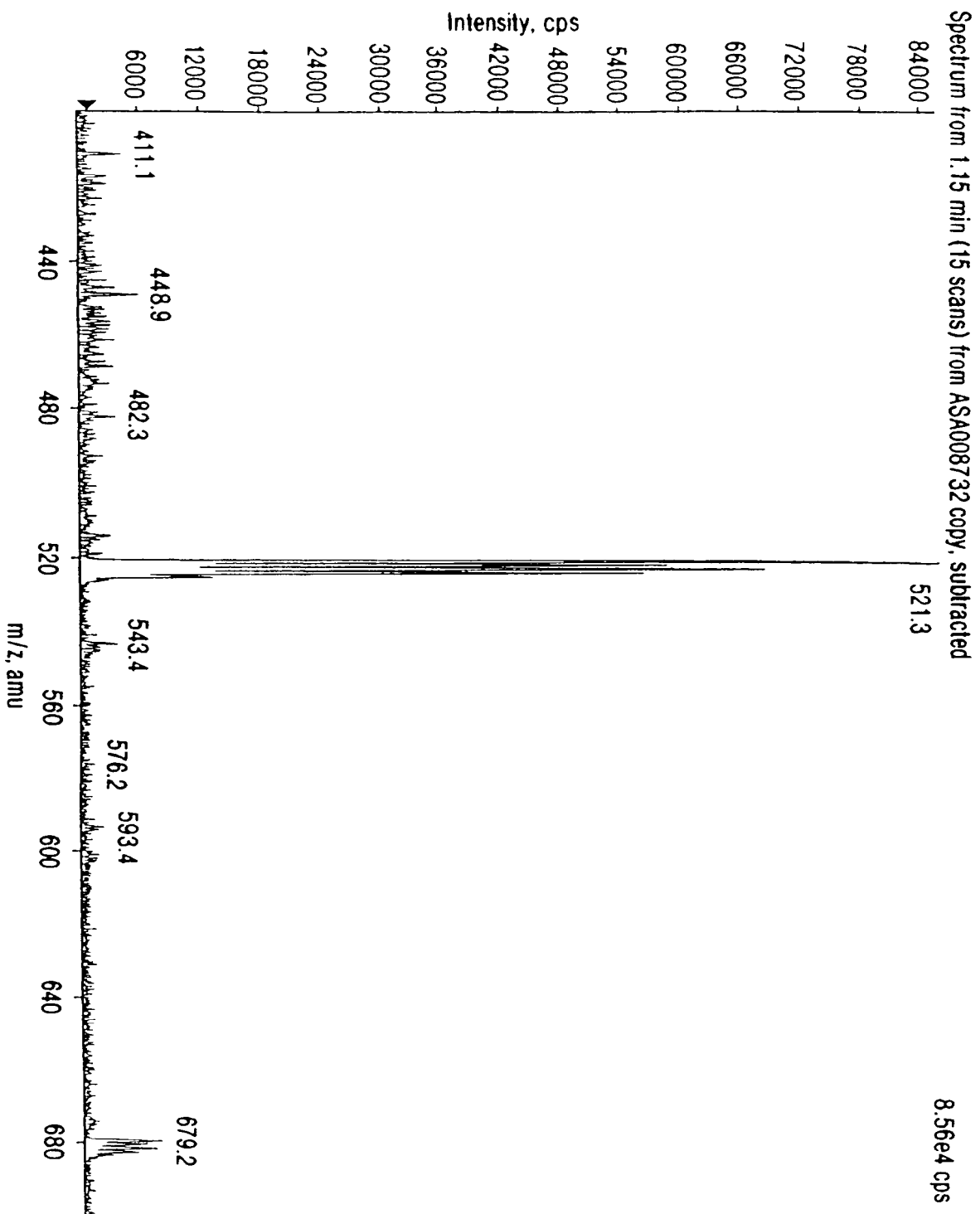


FIG. 178

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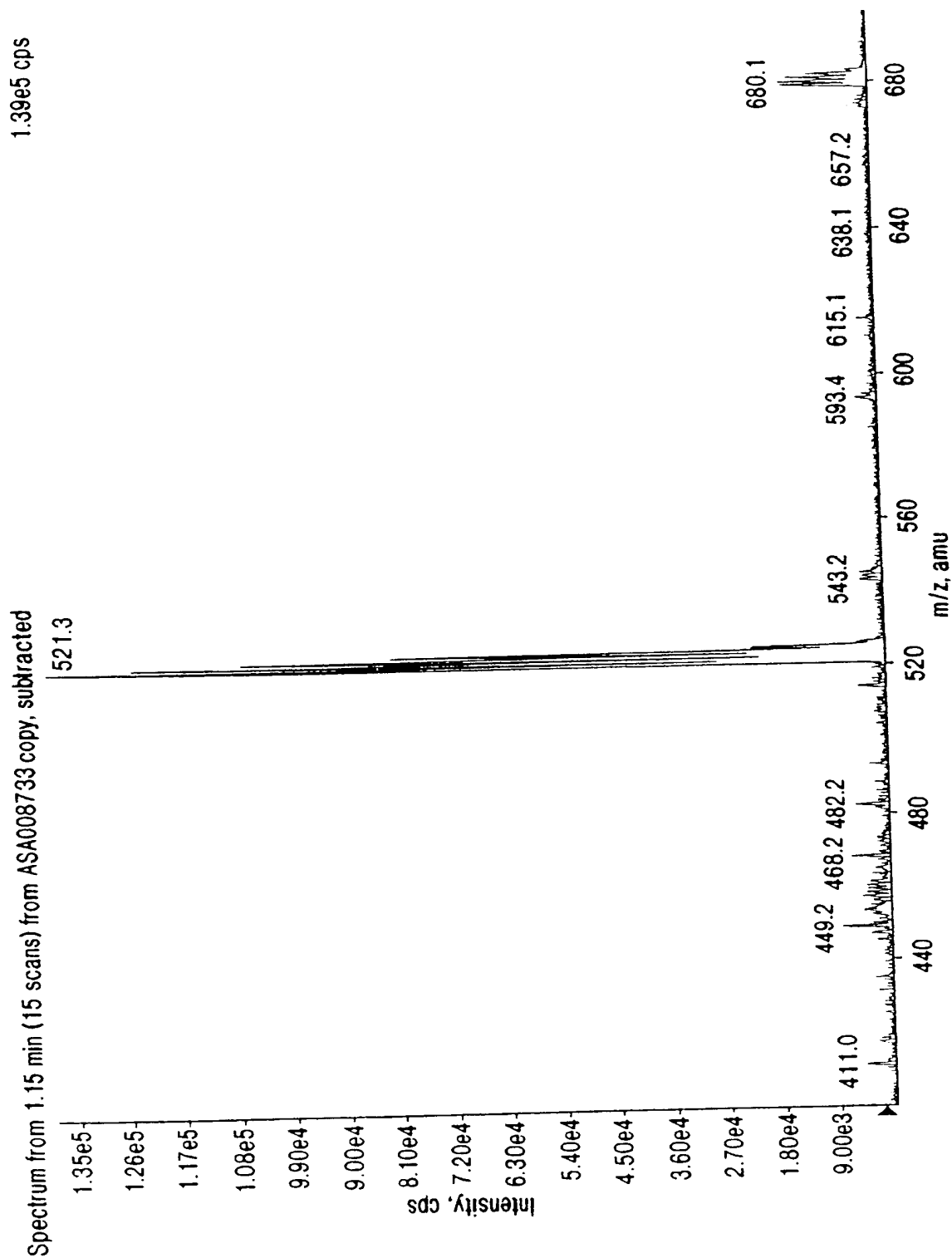


FIG. 179

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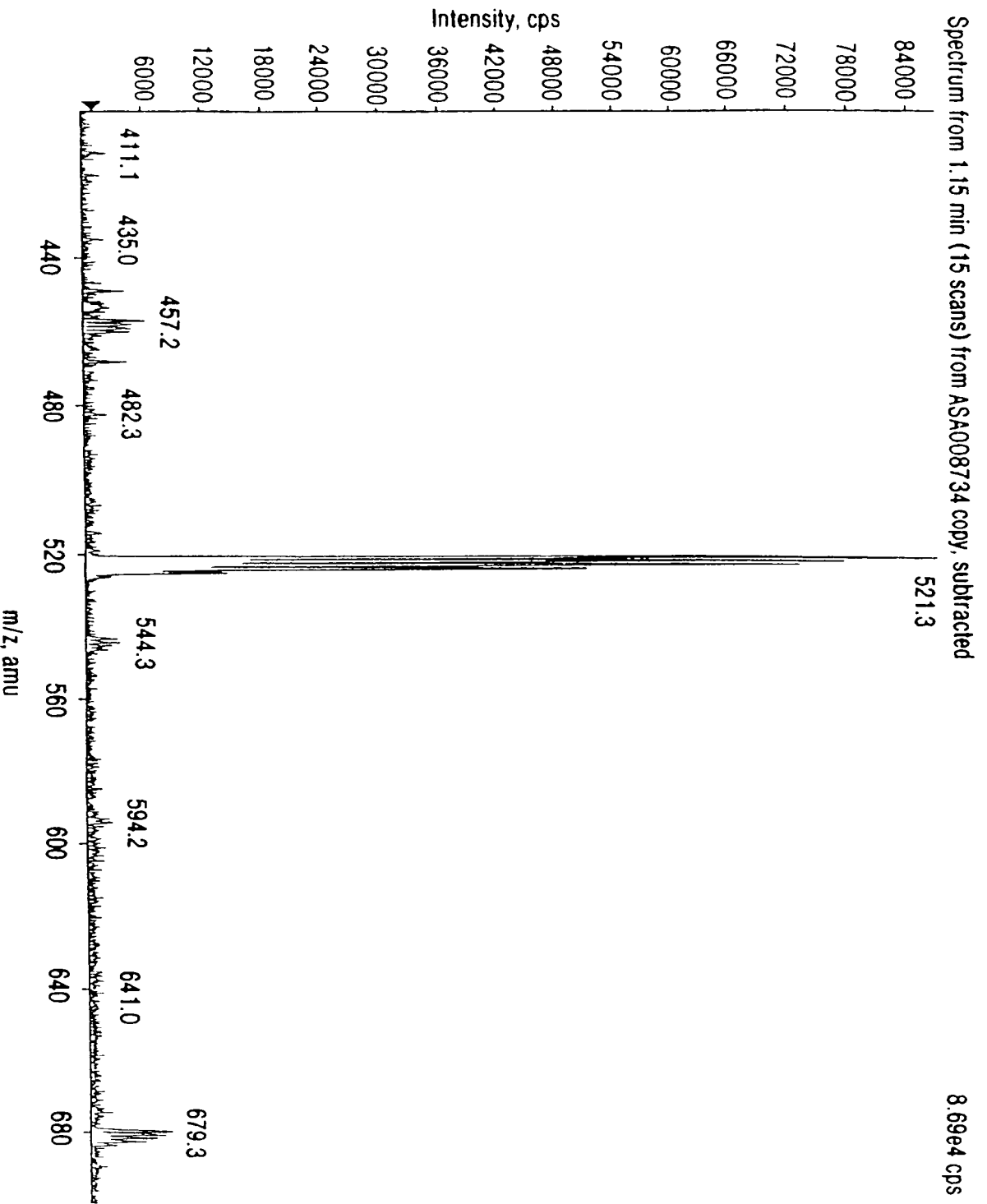


FIG. 180

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7.90e4 cps

Spectrum from 1.15 min (15 scans) from ASA008735 copy, subtracted

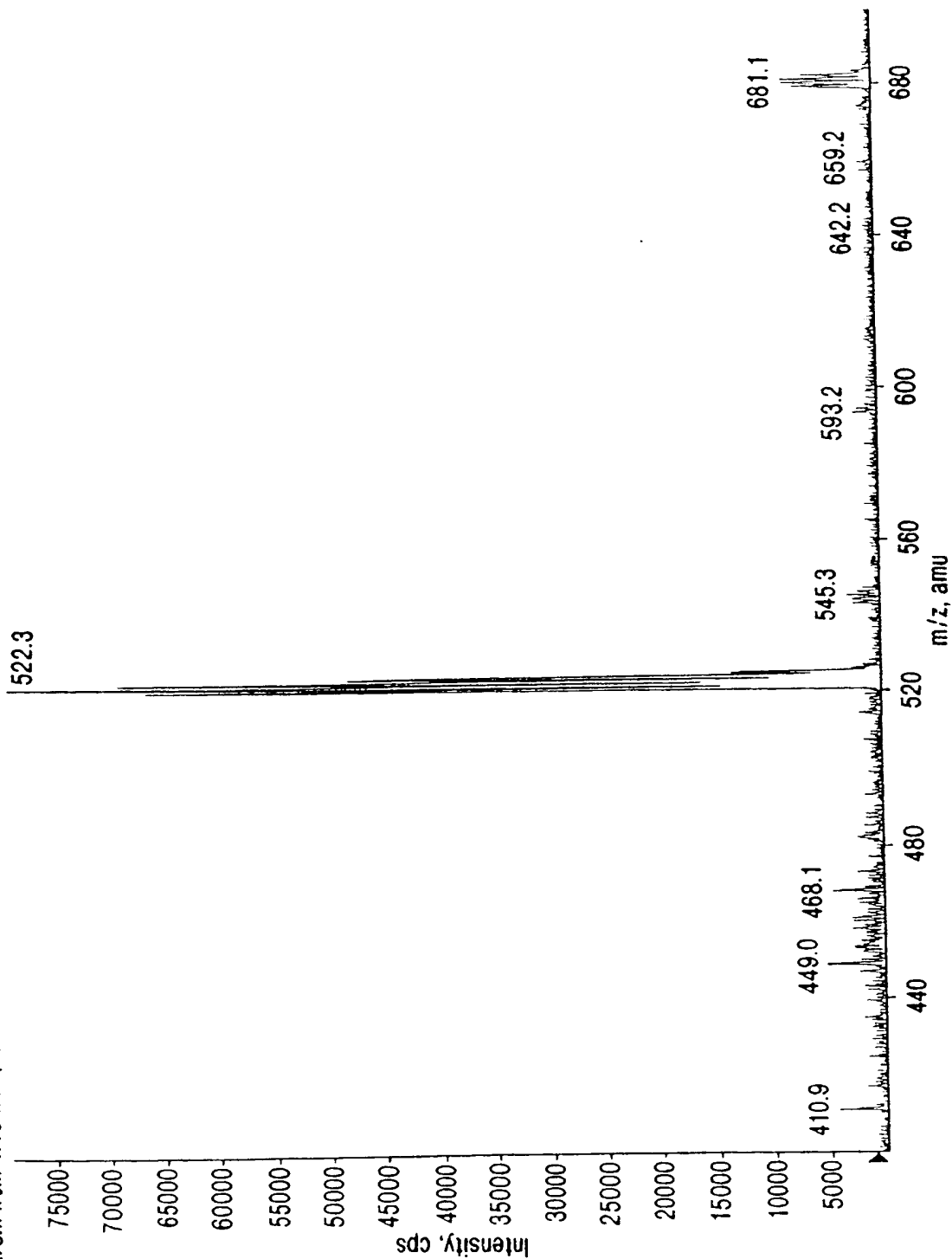


FIG. 181

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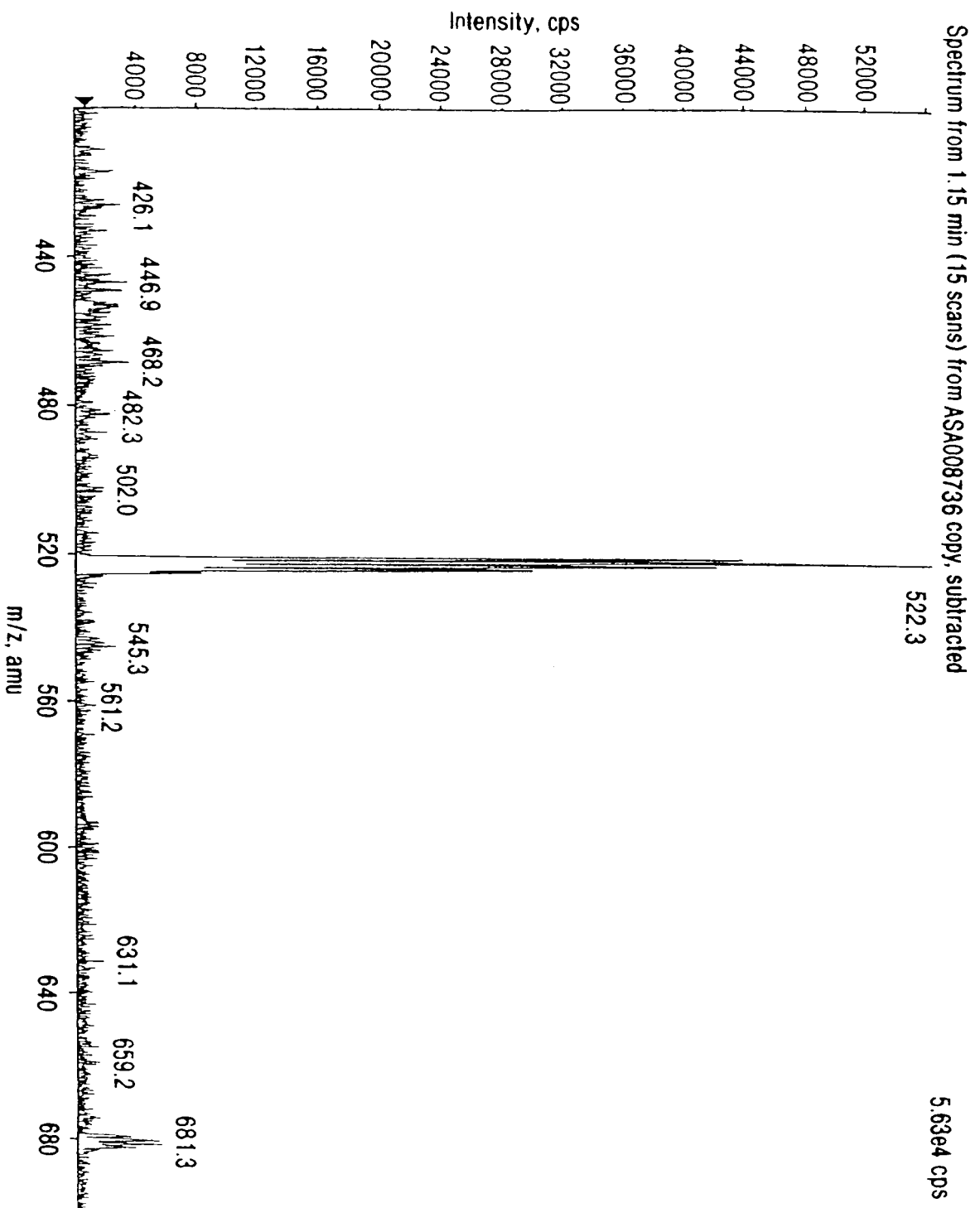


FIG. 182

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7.51e4 cps

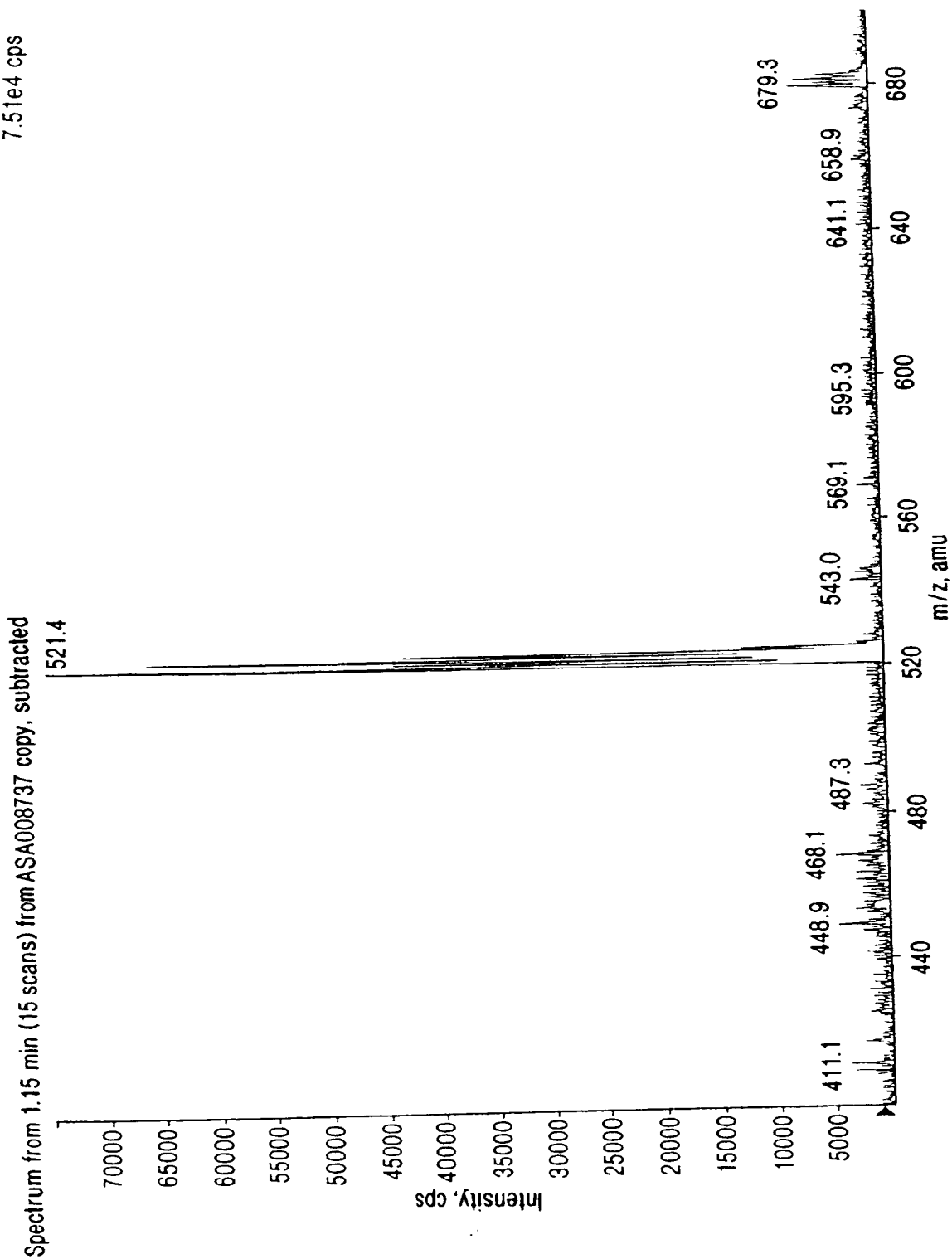


FIG. 183

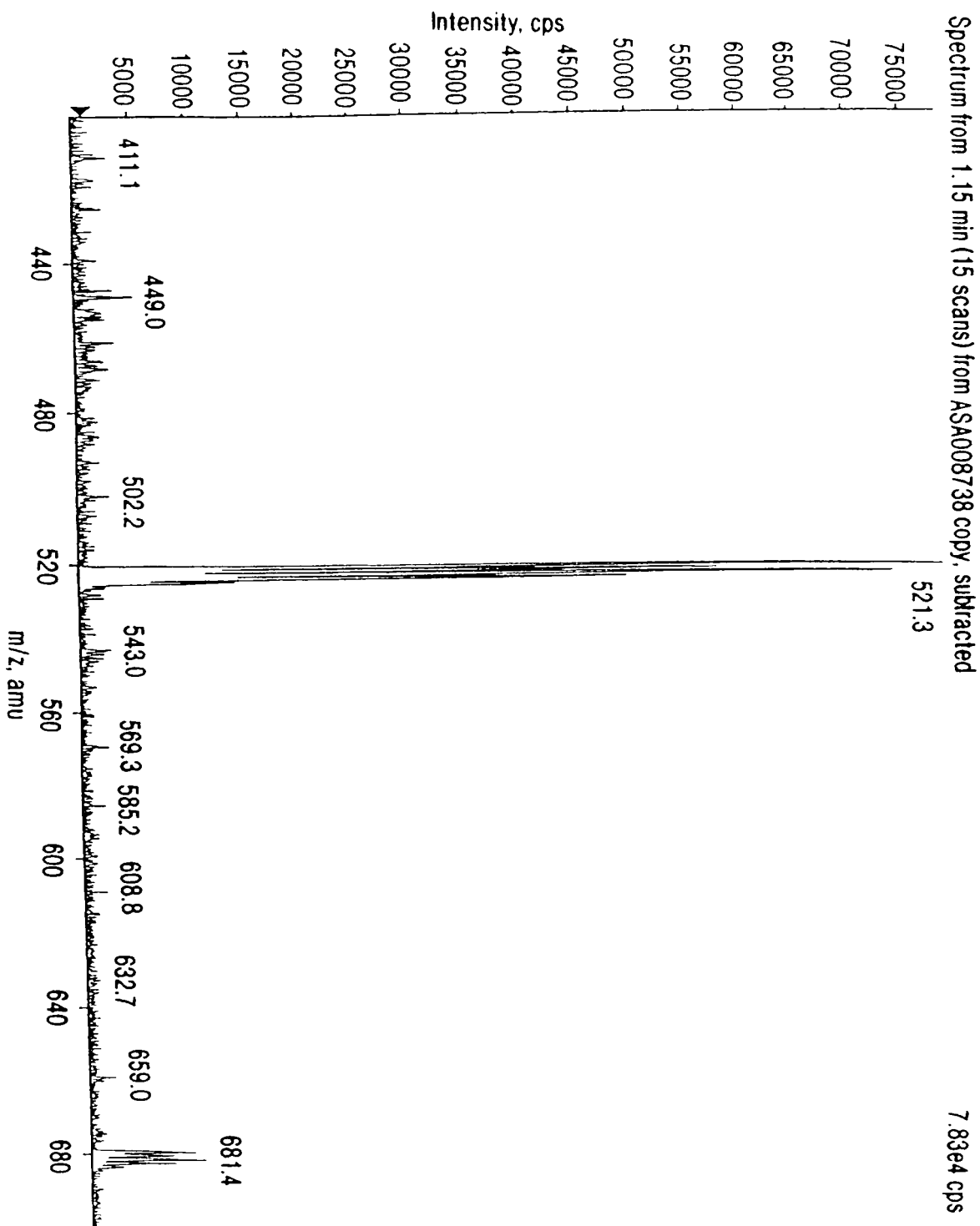


FIG. 184

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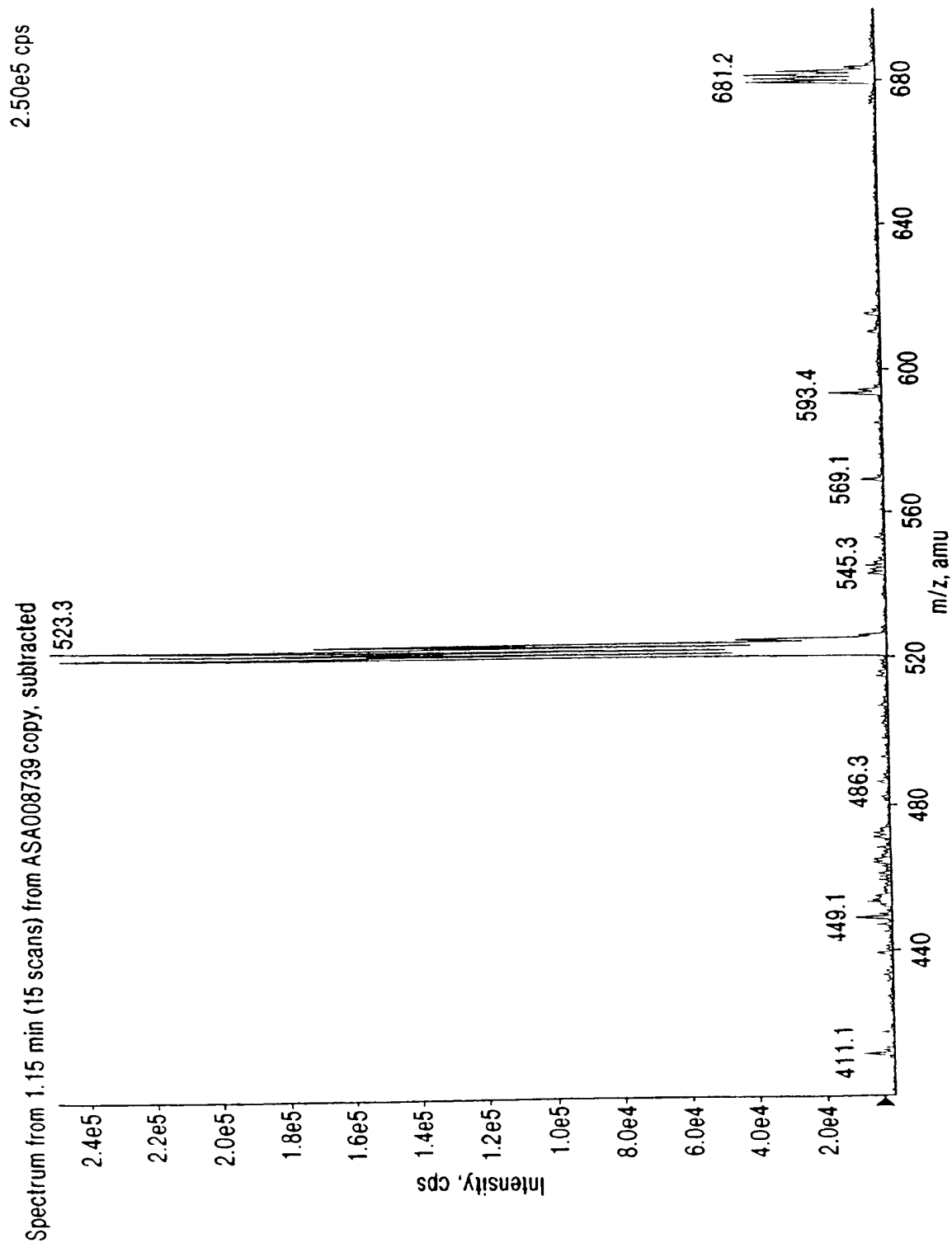


FIG. 185

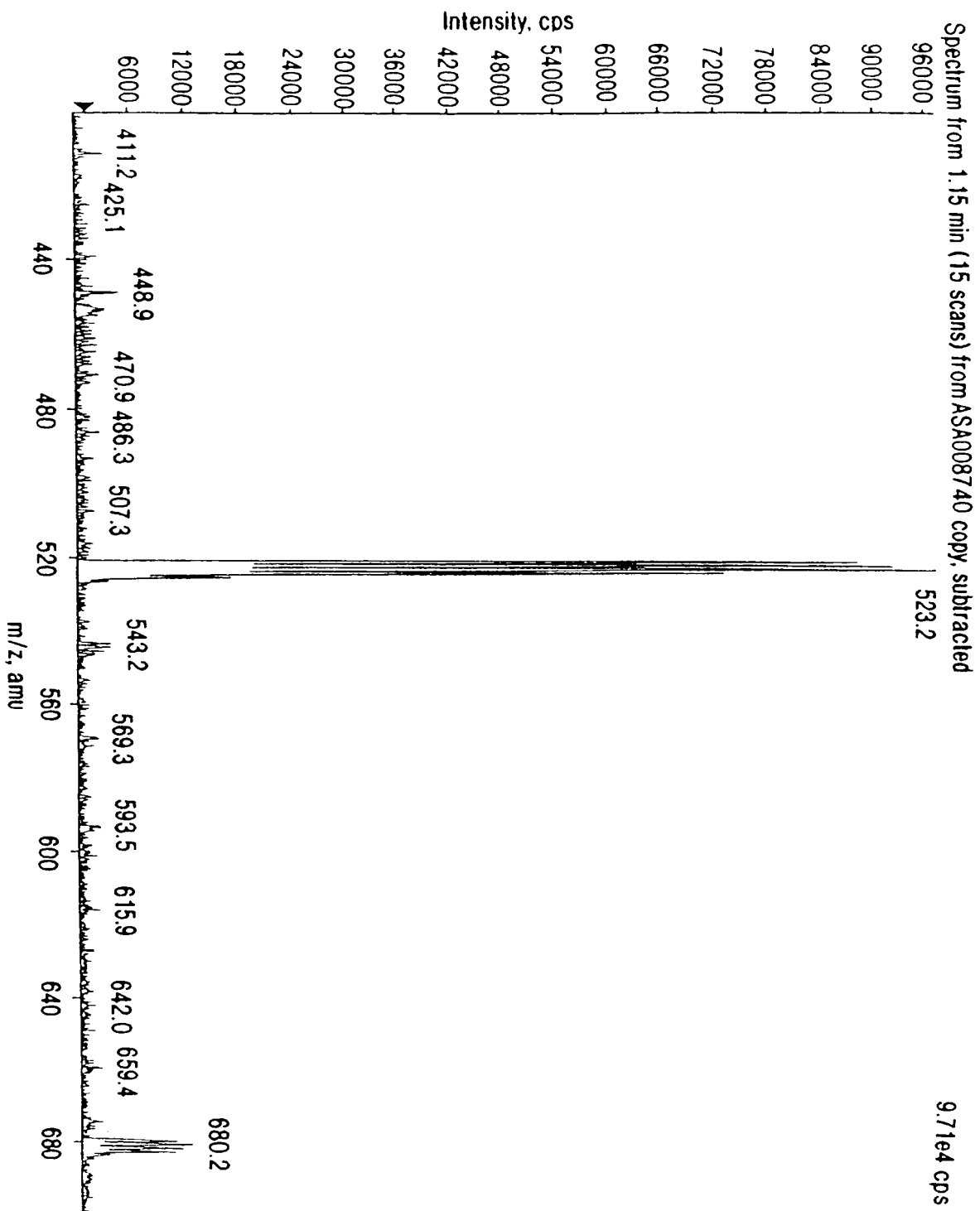


FIG. 186

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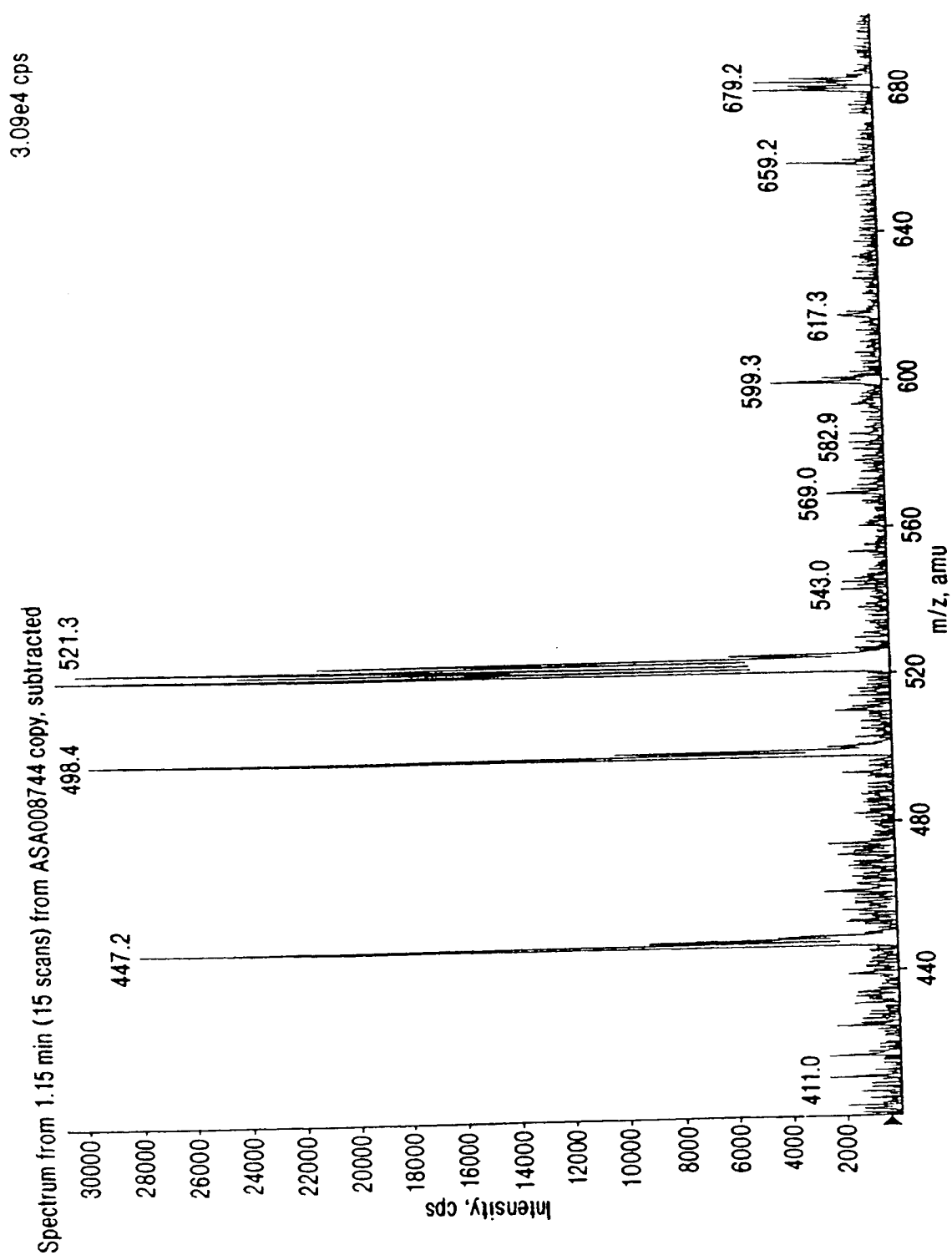


FIG. 187

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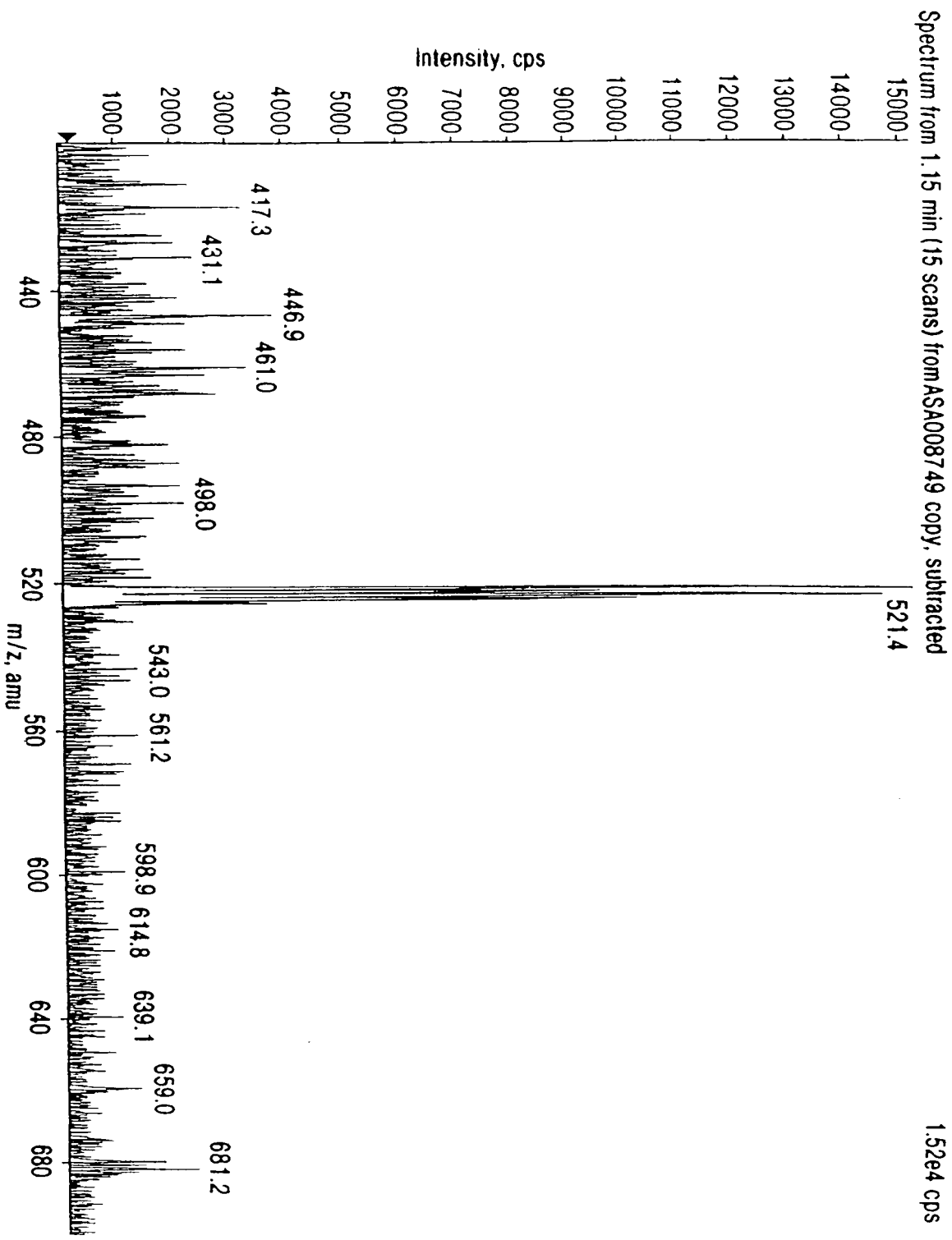


FIG. 188

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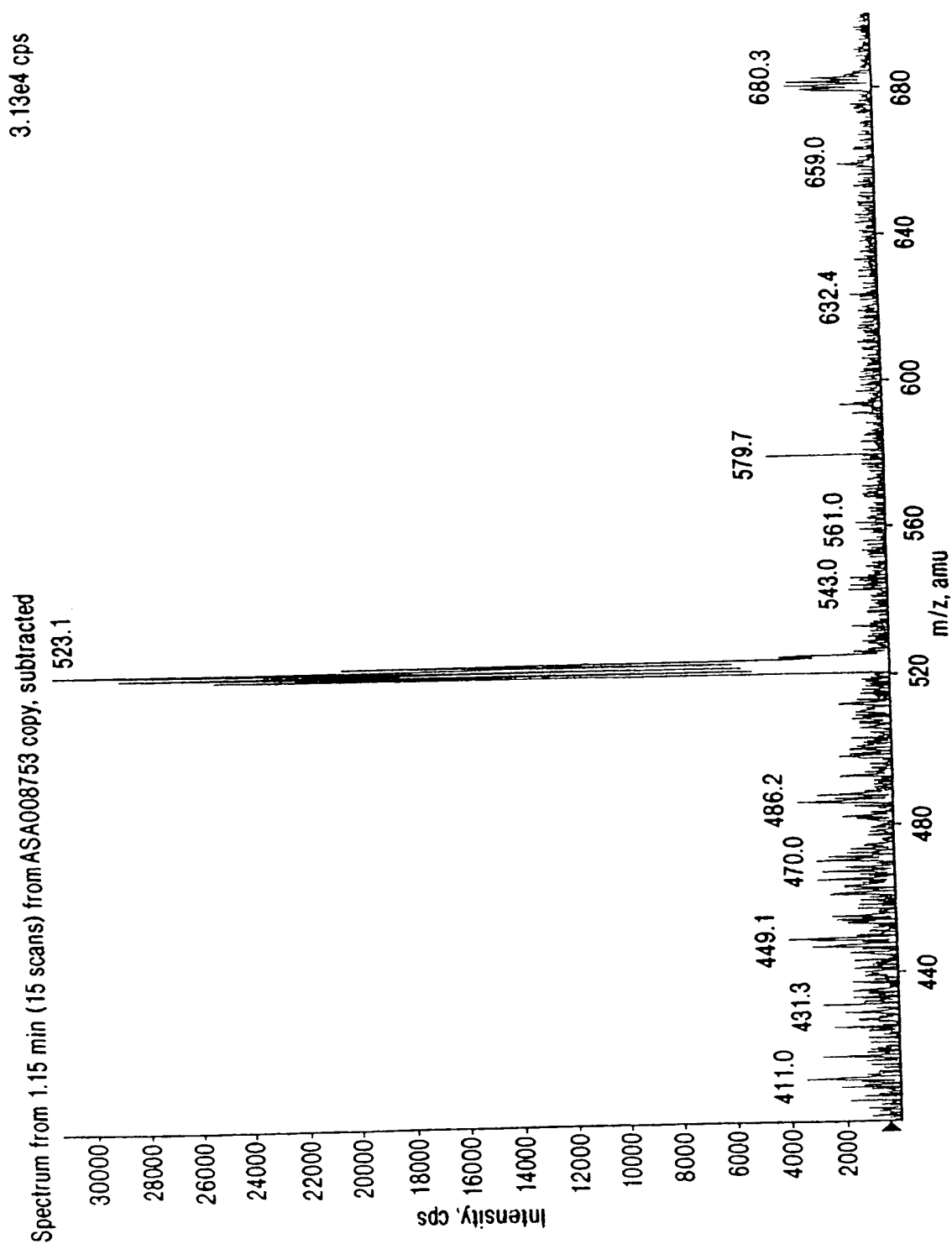


FIG. 189

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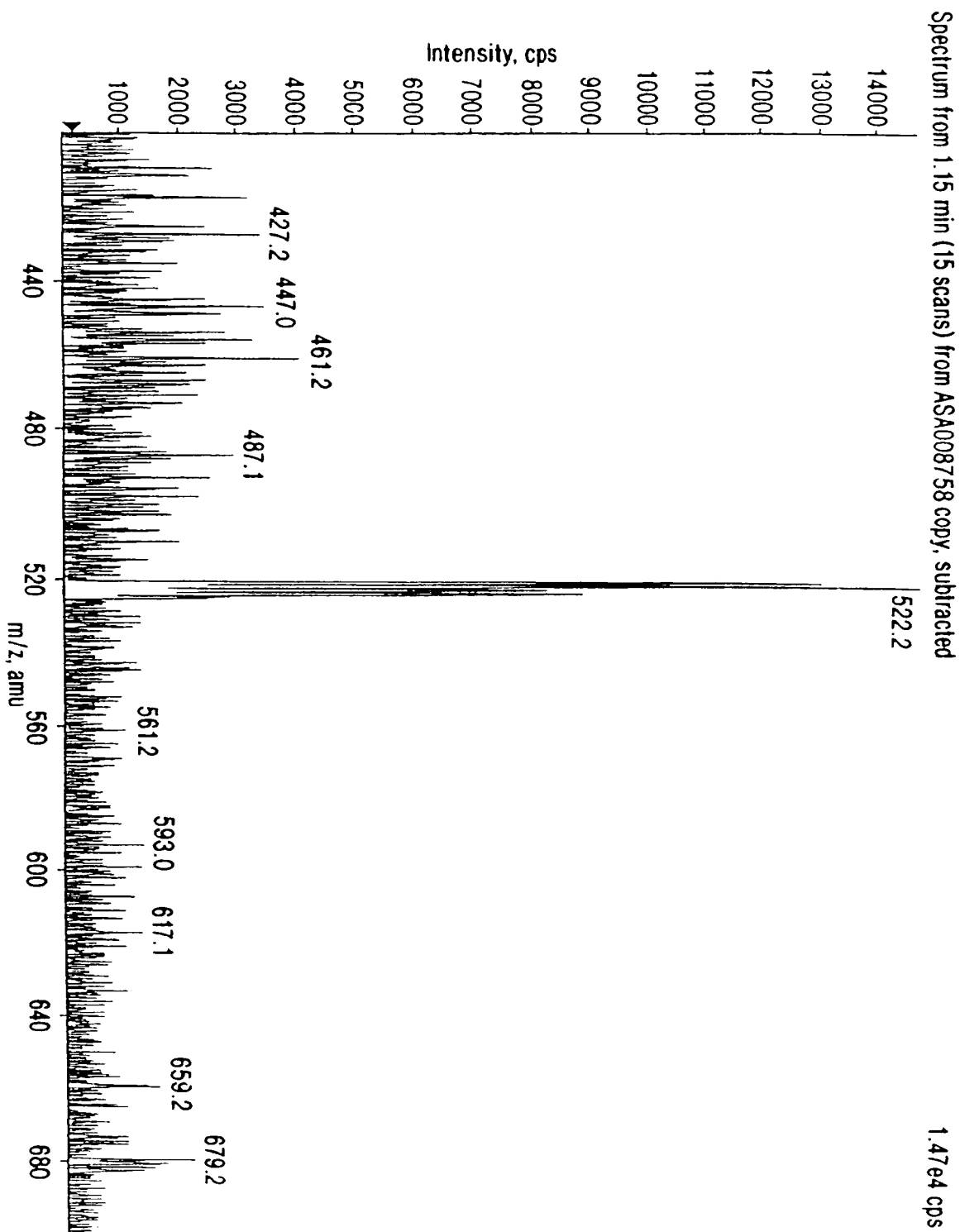


FIG. 190

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6.87e3 cps

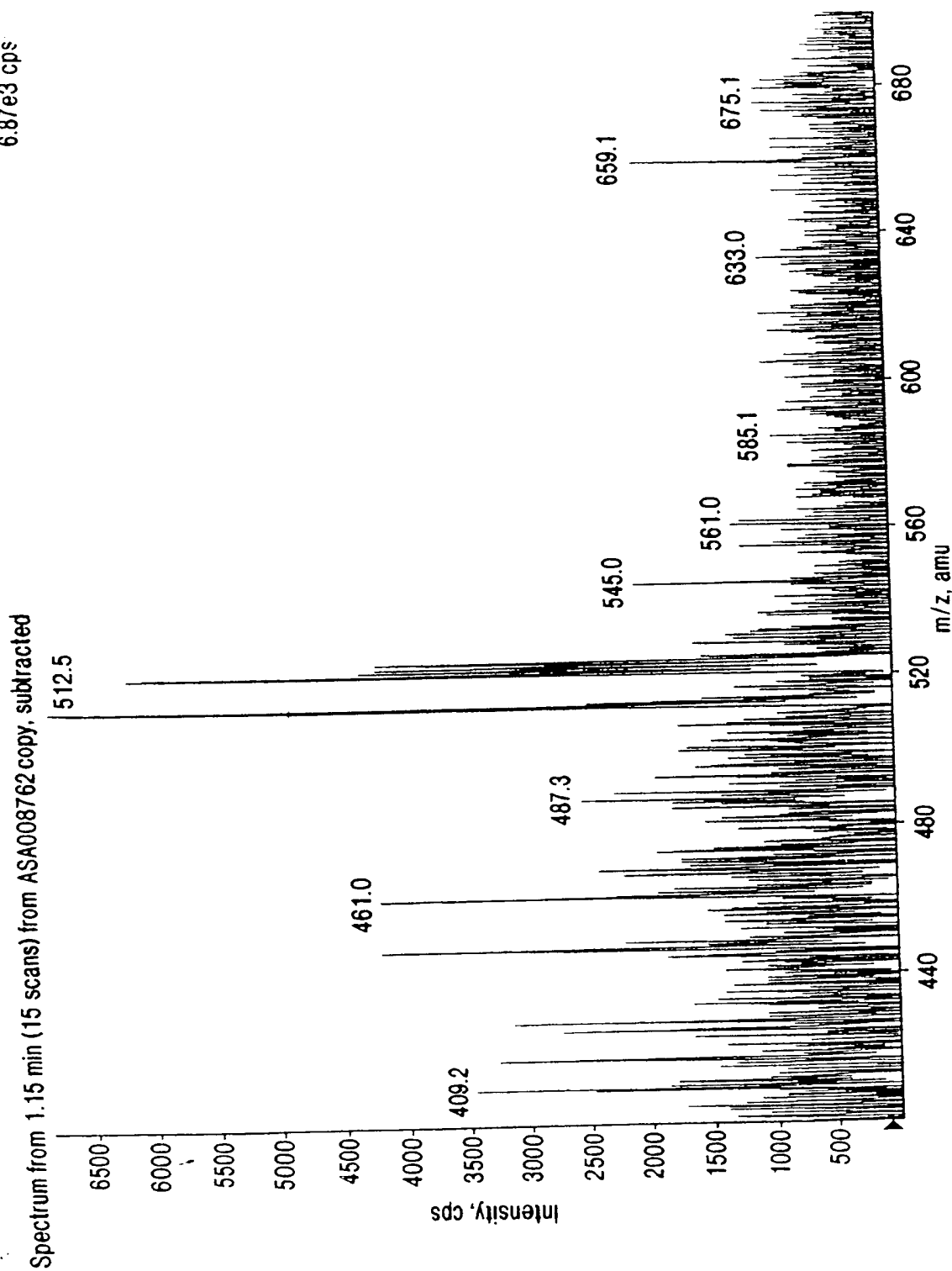


FIG. 191

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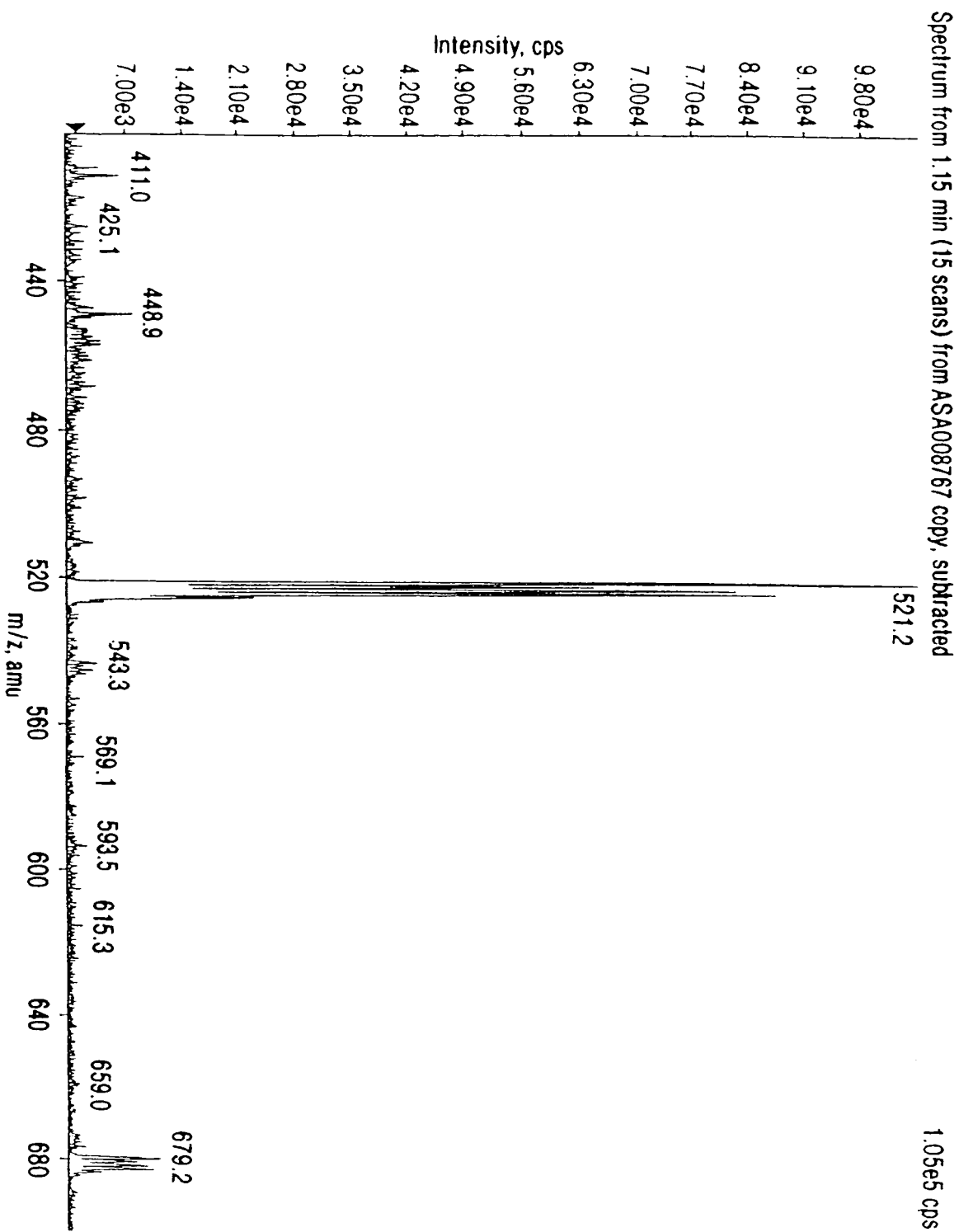


FIG. 192

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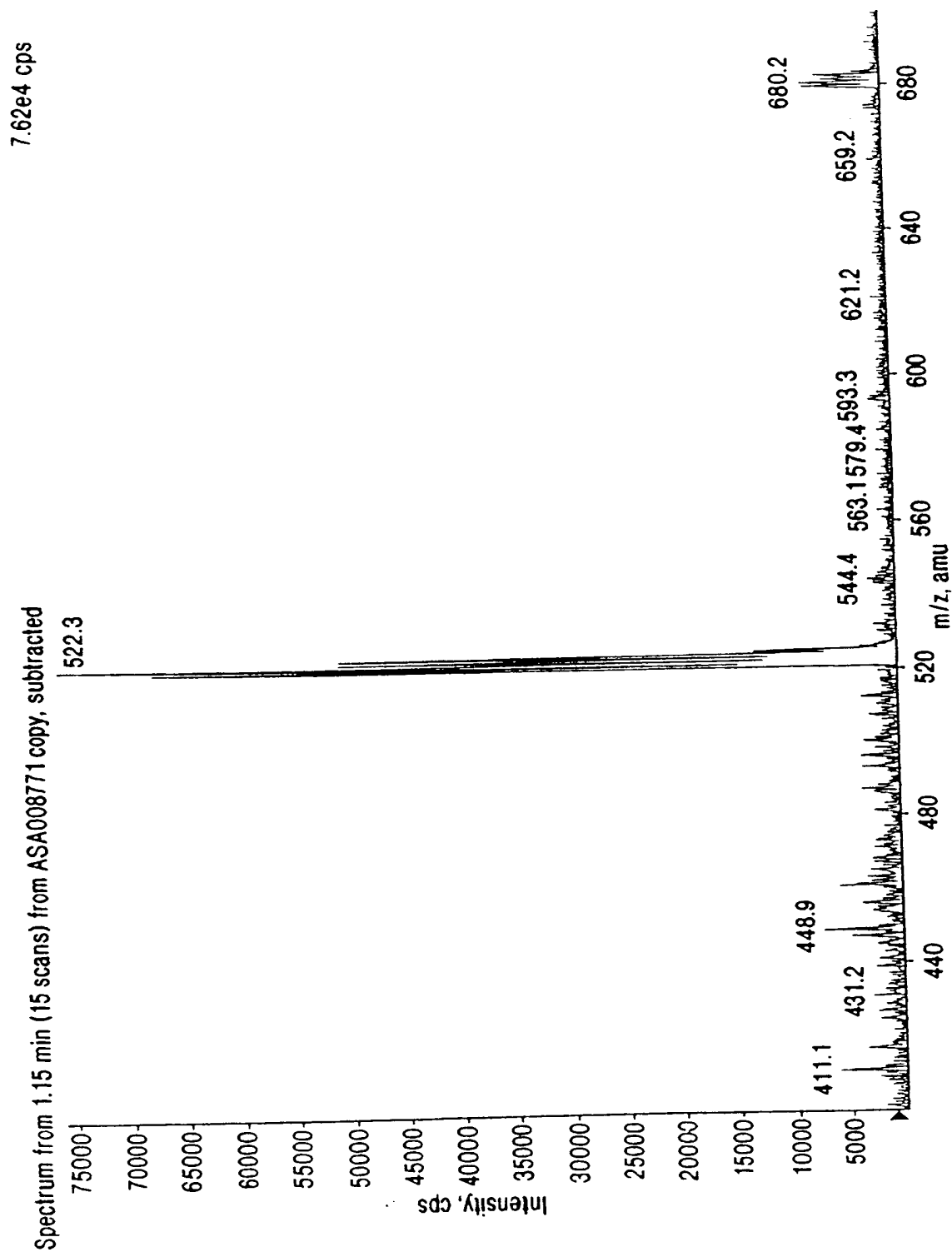


FIG. 193

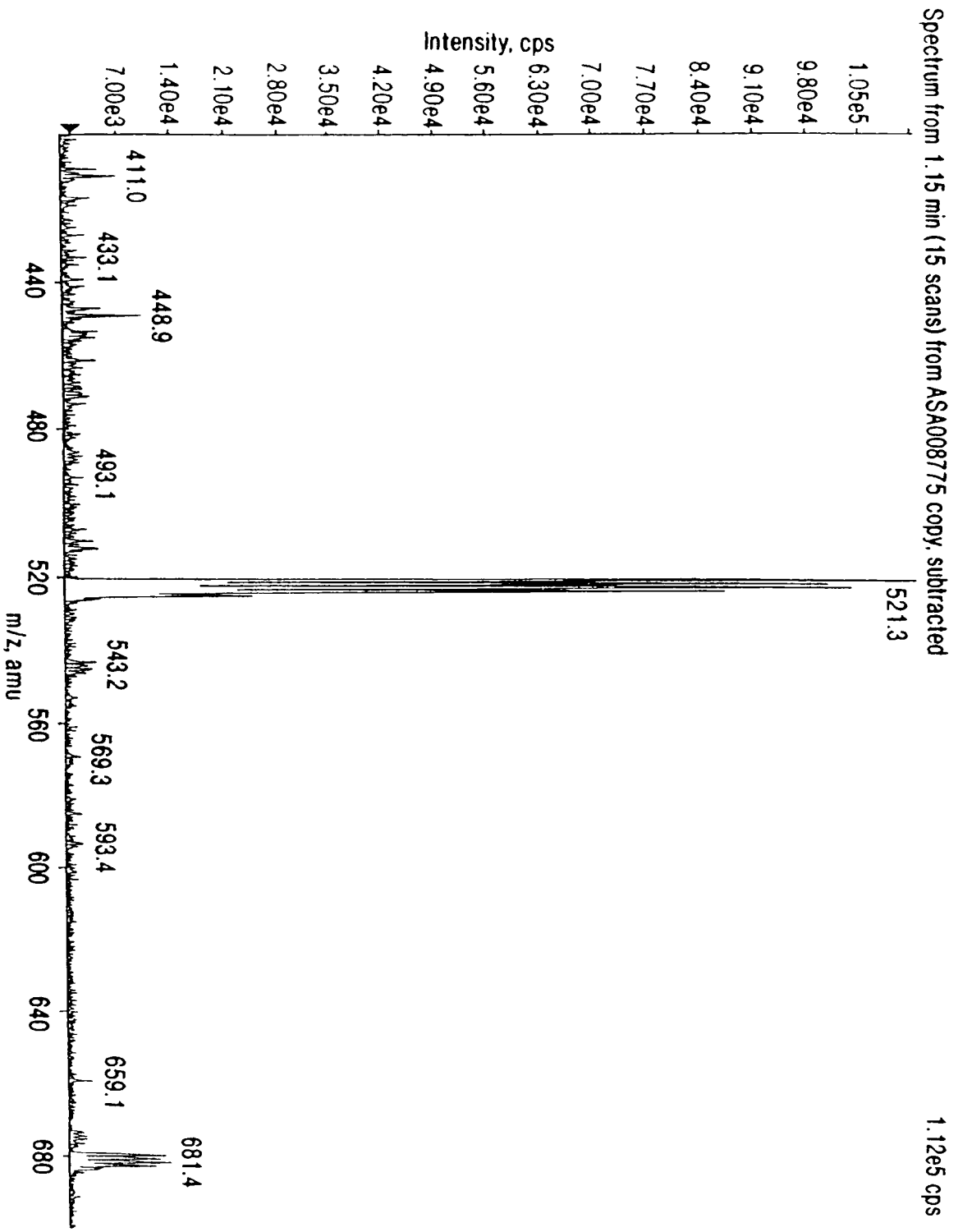


FIG. 194

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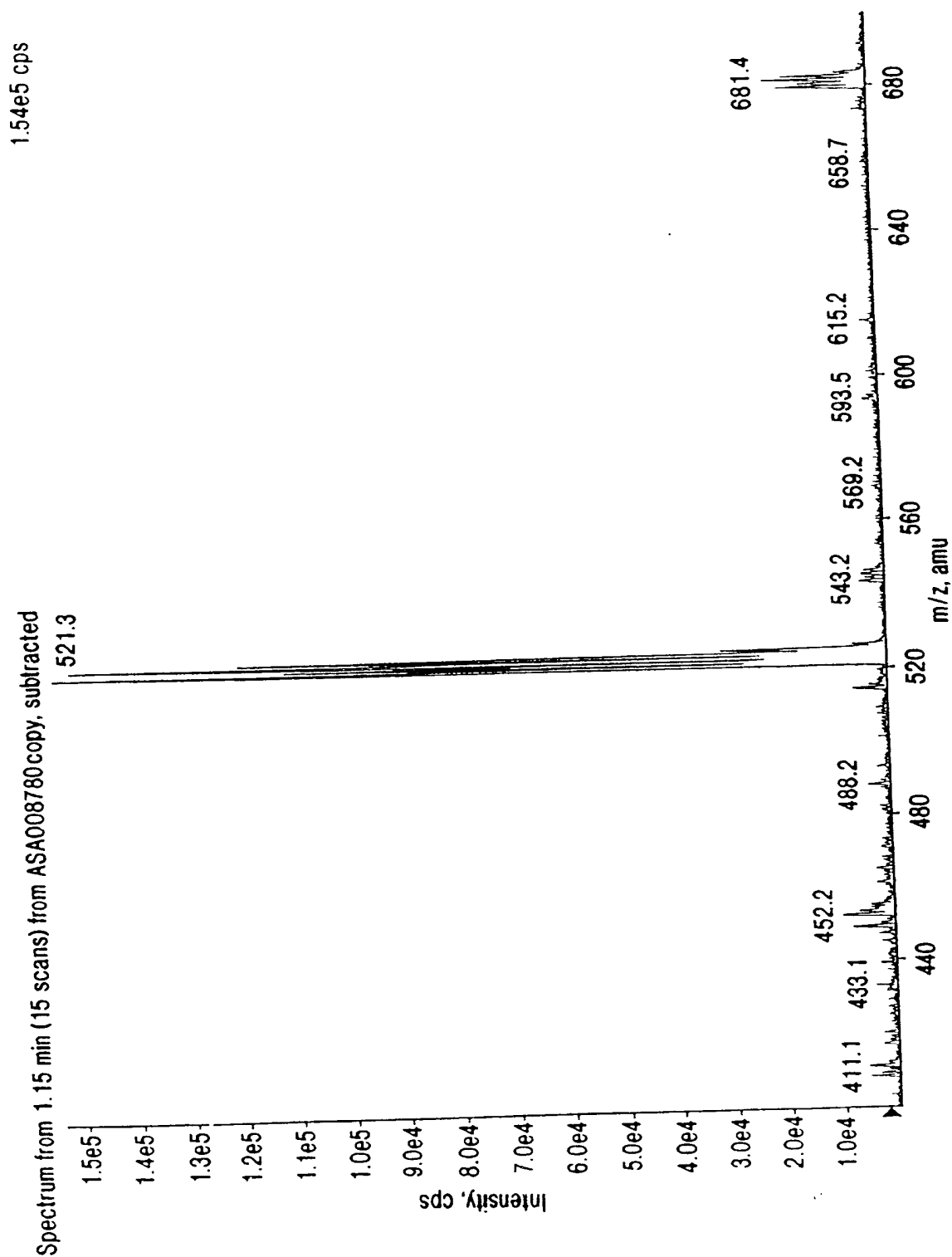


FIG. 195

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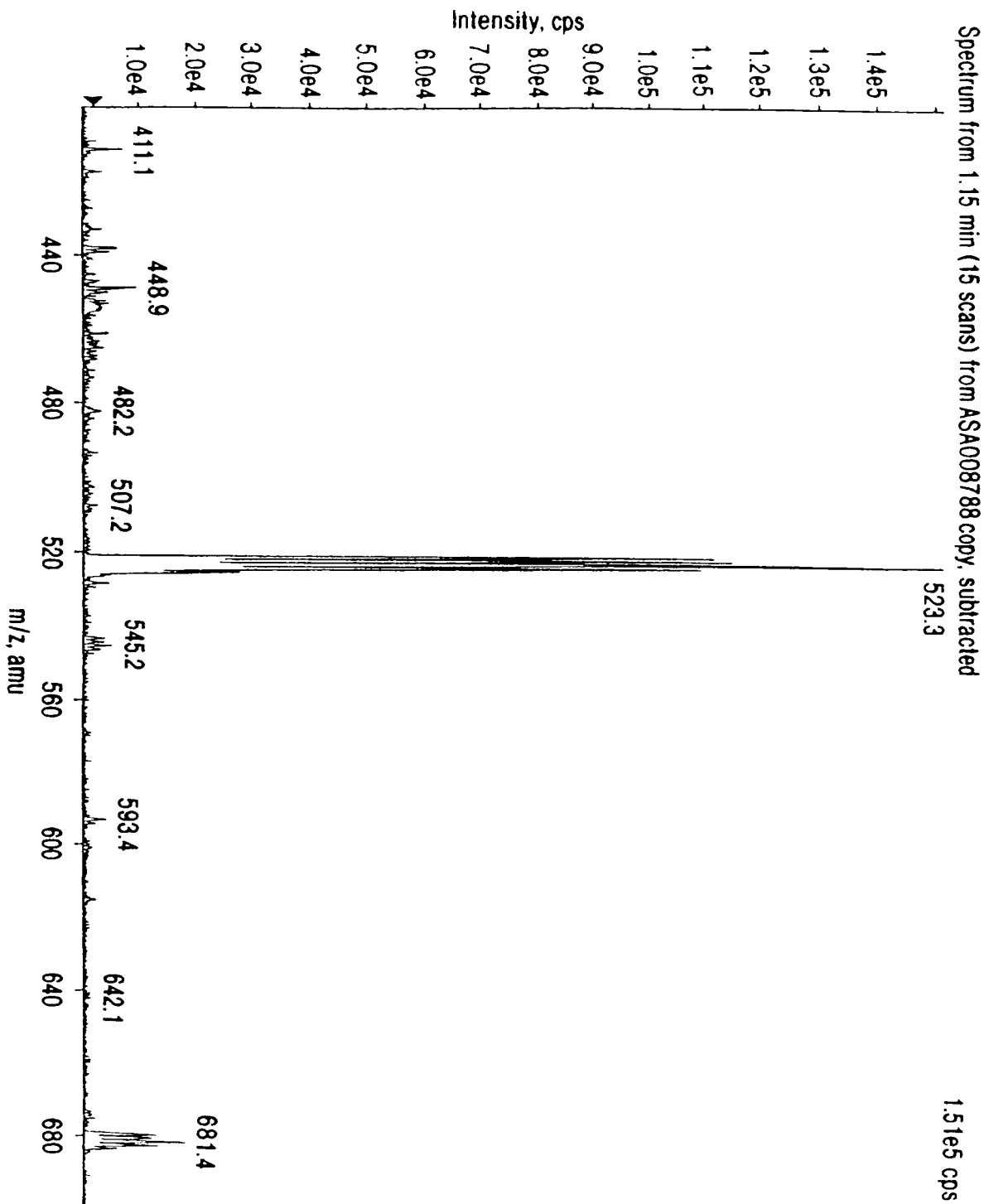


FIG. 196

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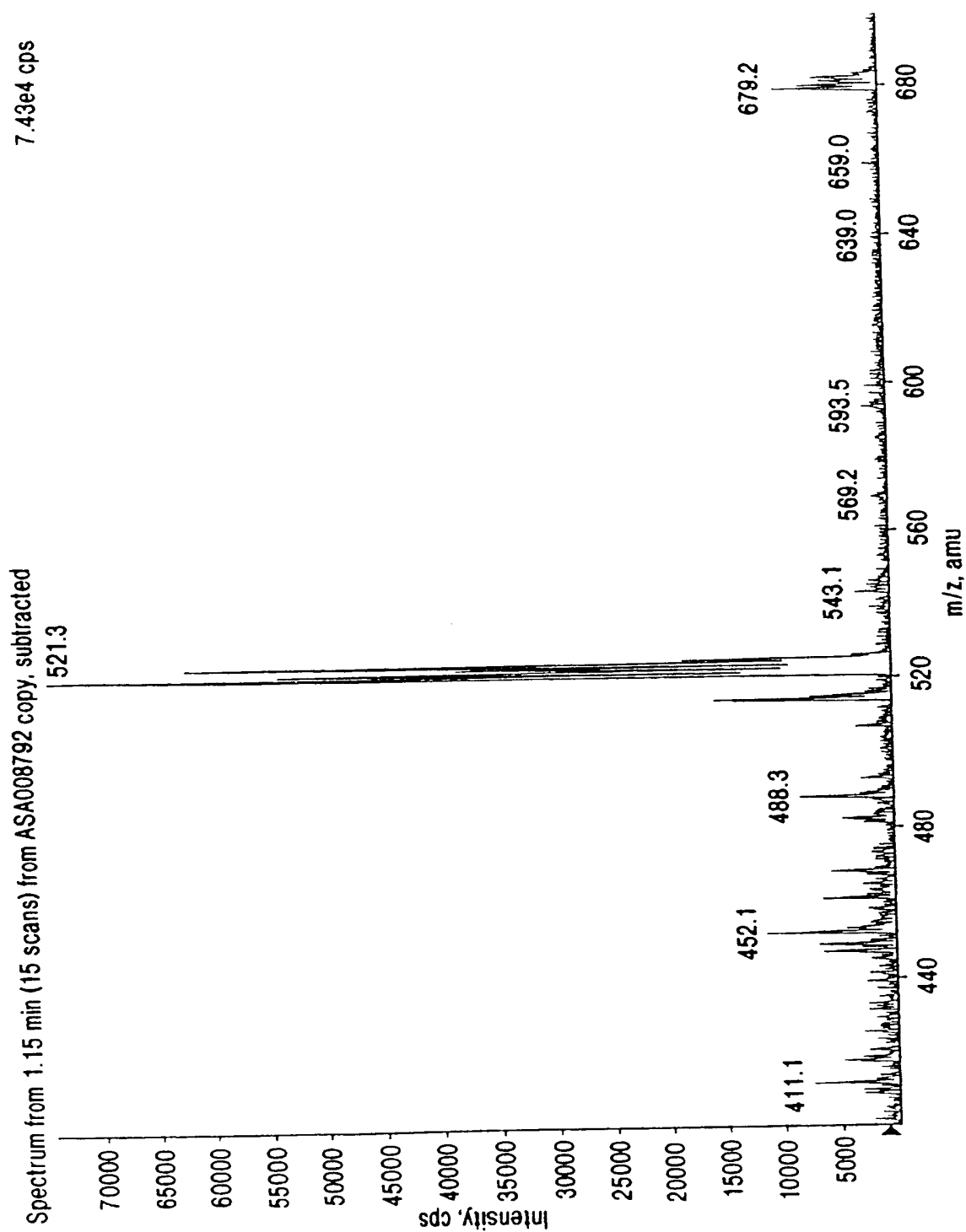


FIG. 197

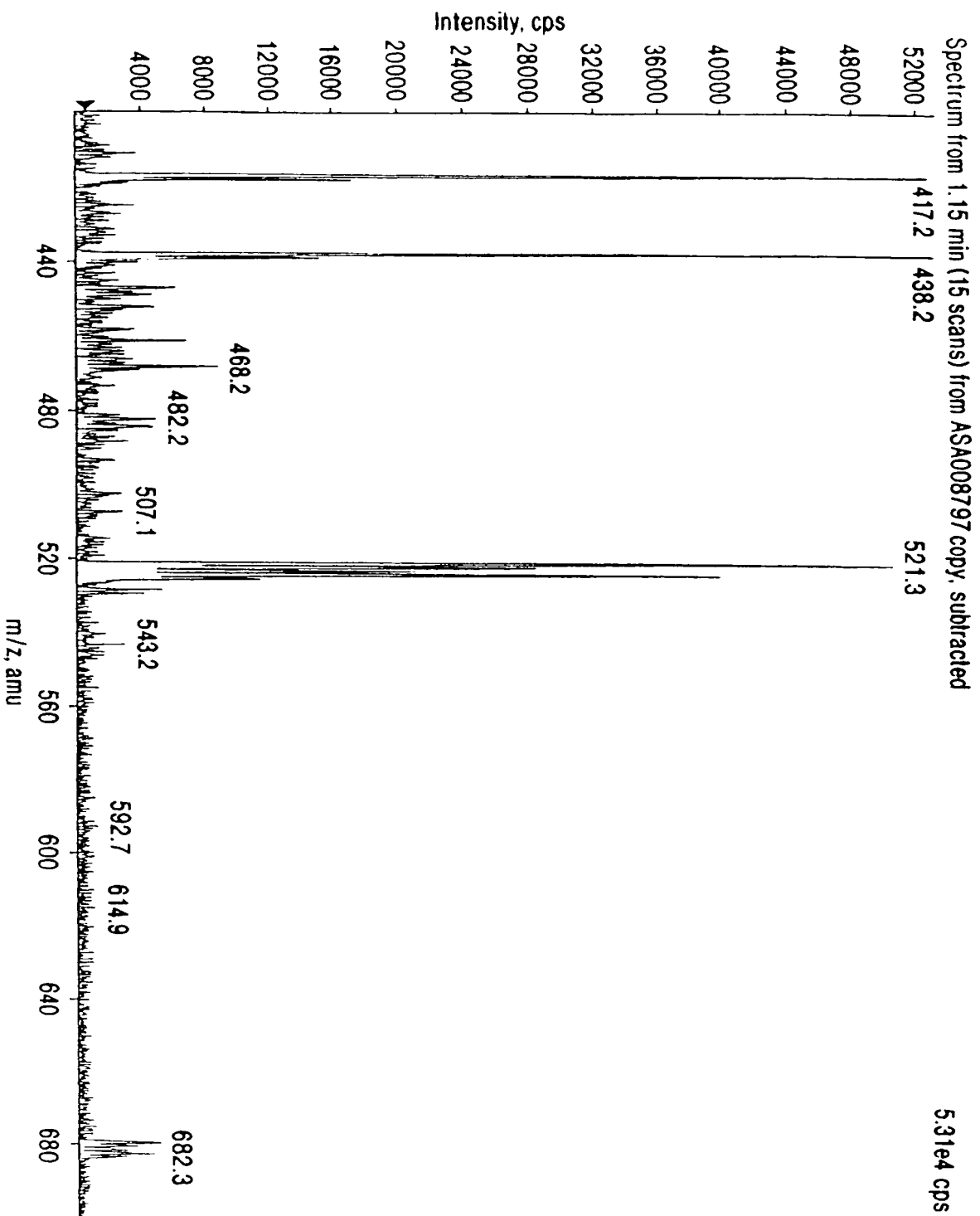


FIG. 198

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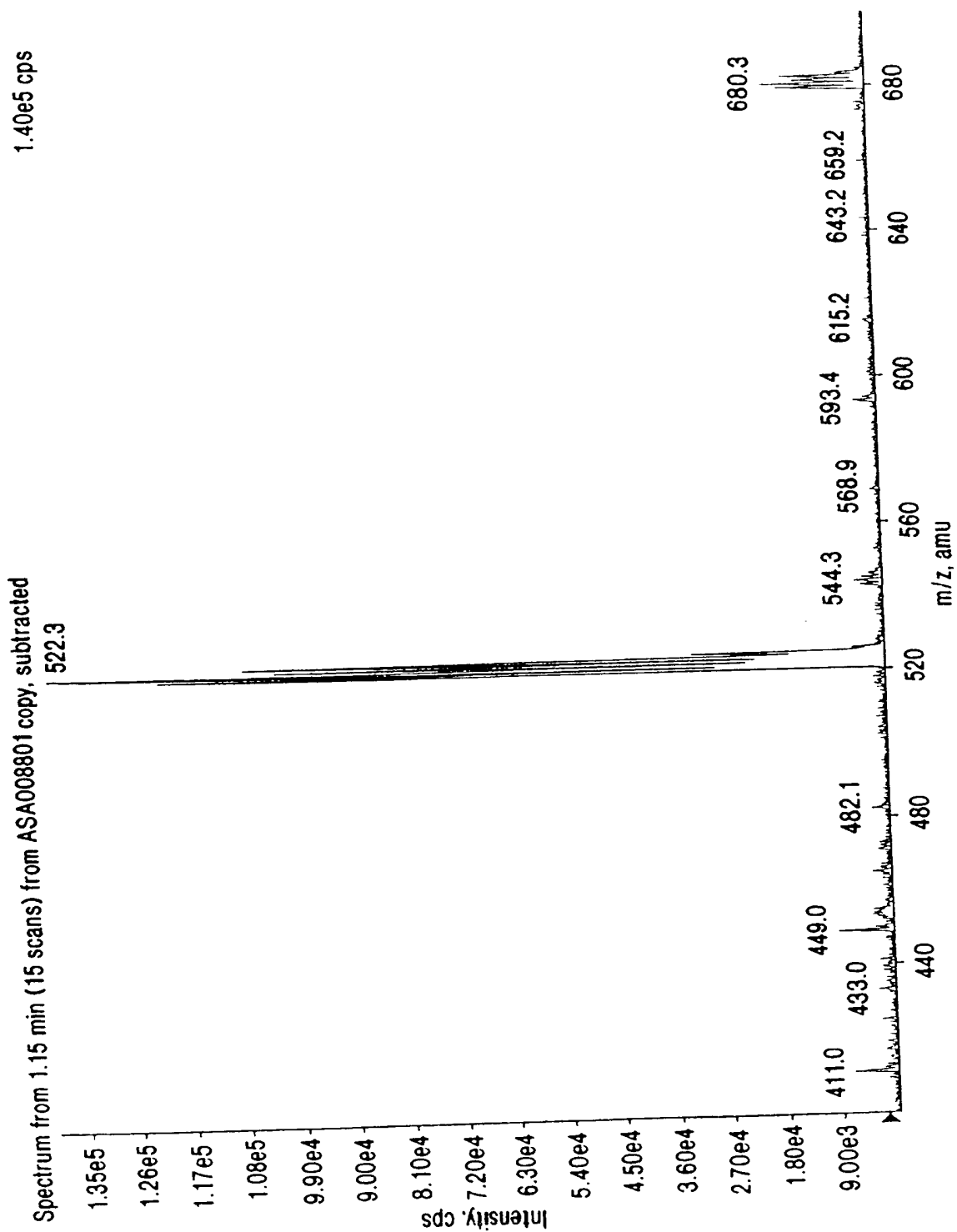


FIG. 199

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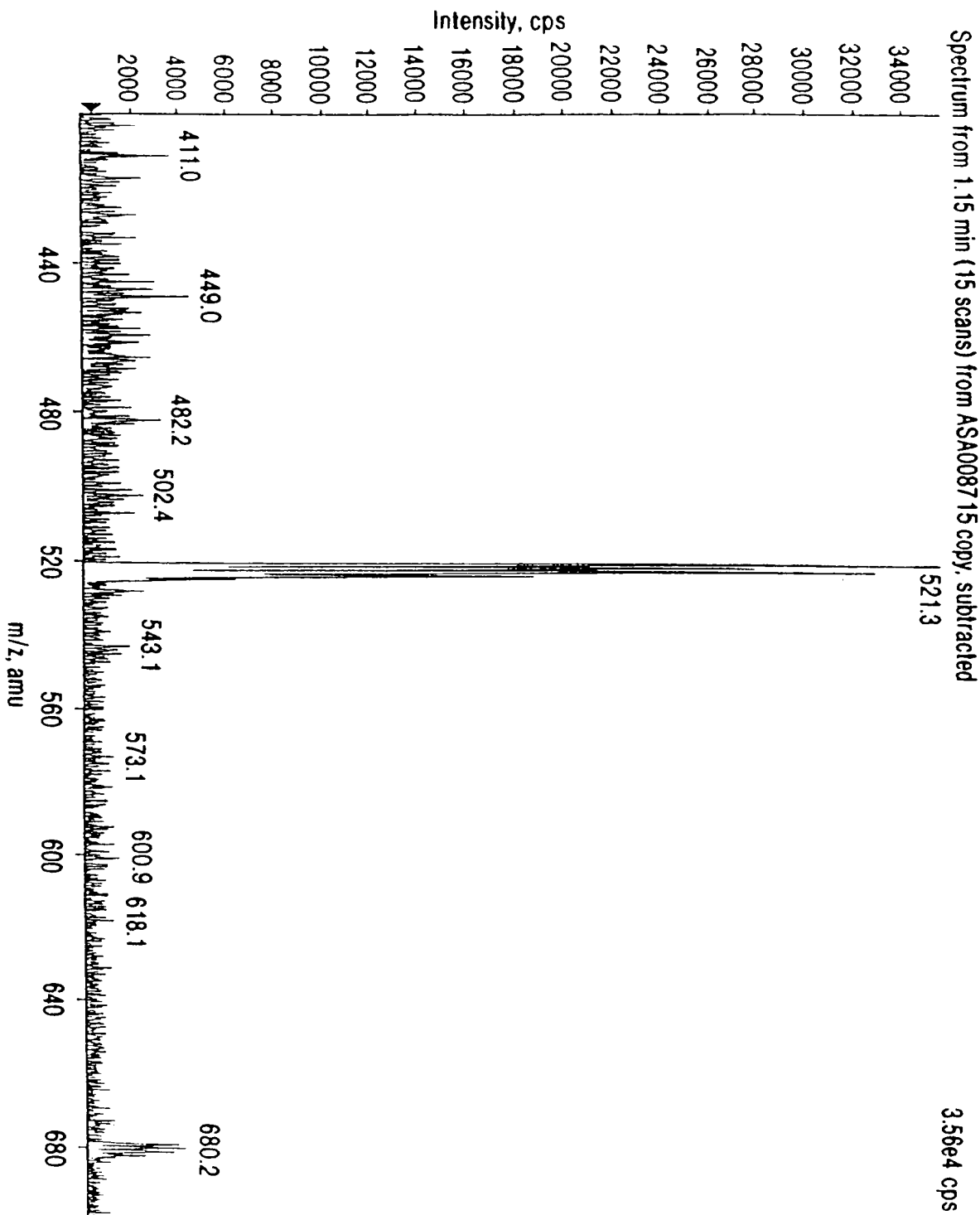


FIG. 200

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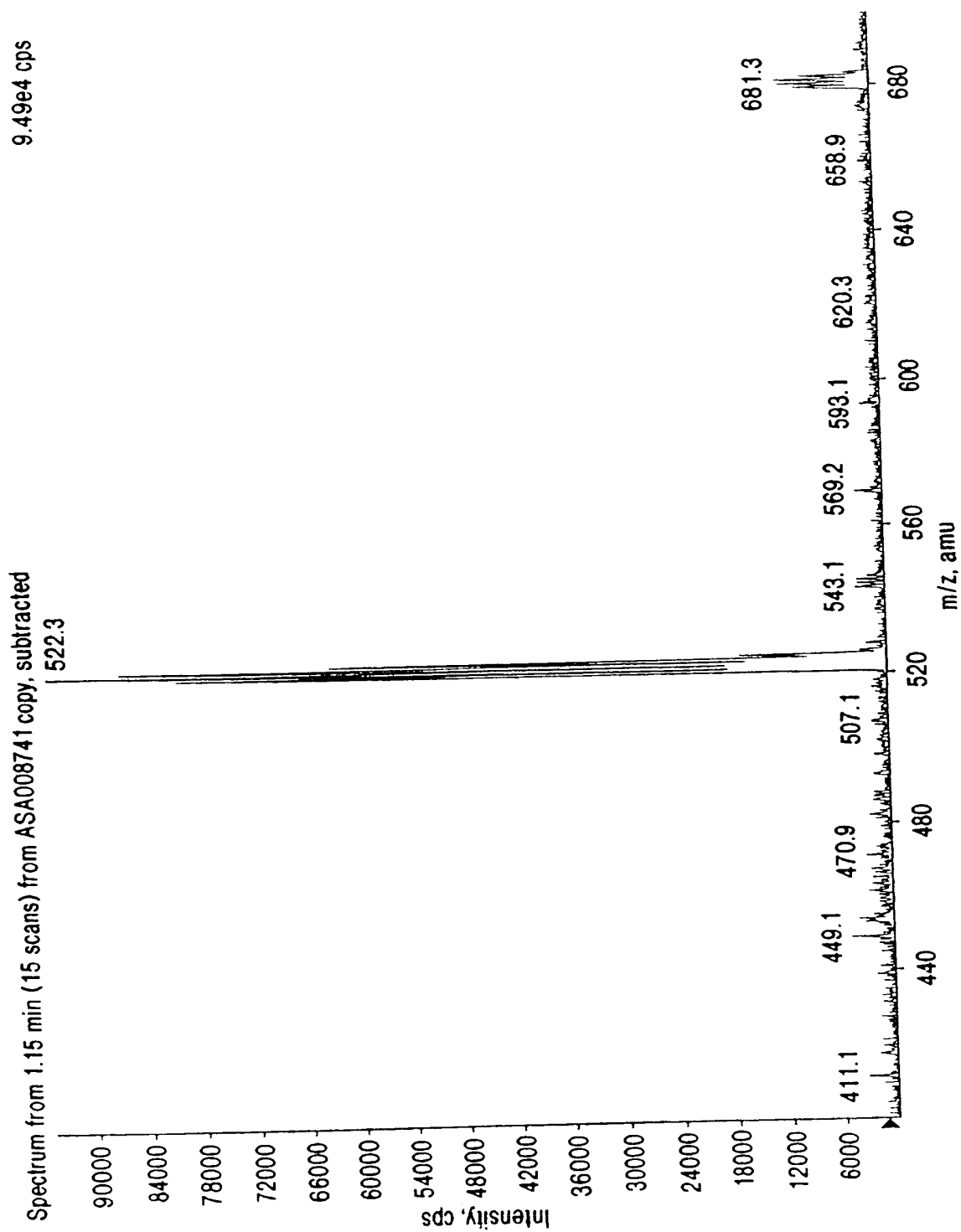


FIG. 201

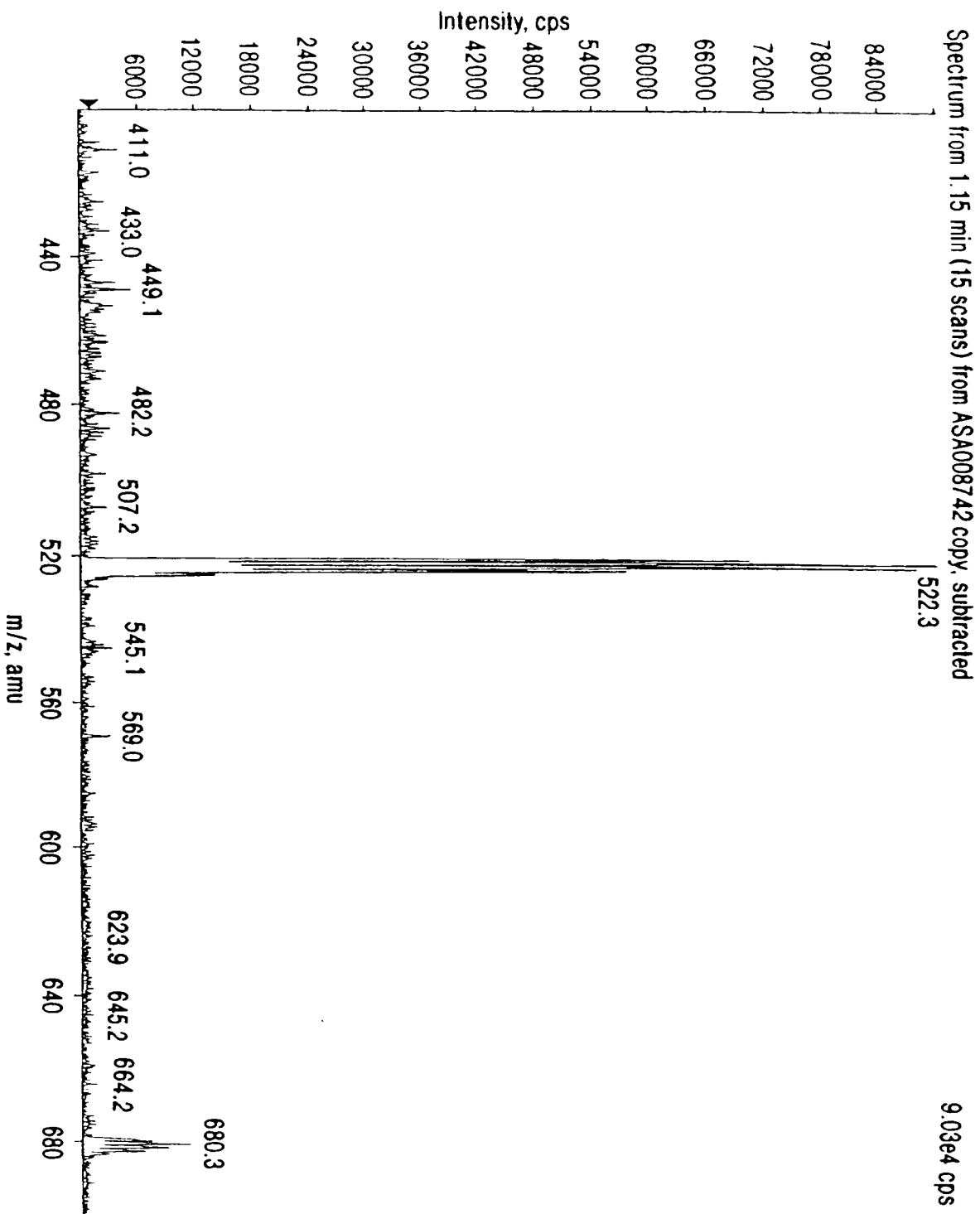


FIG. 202

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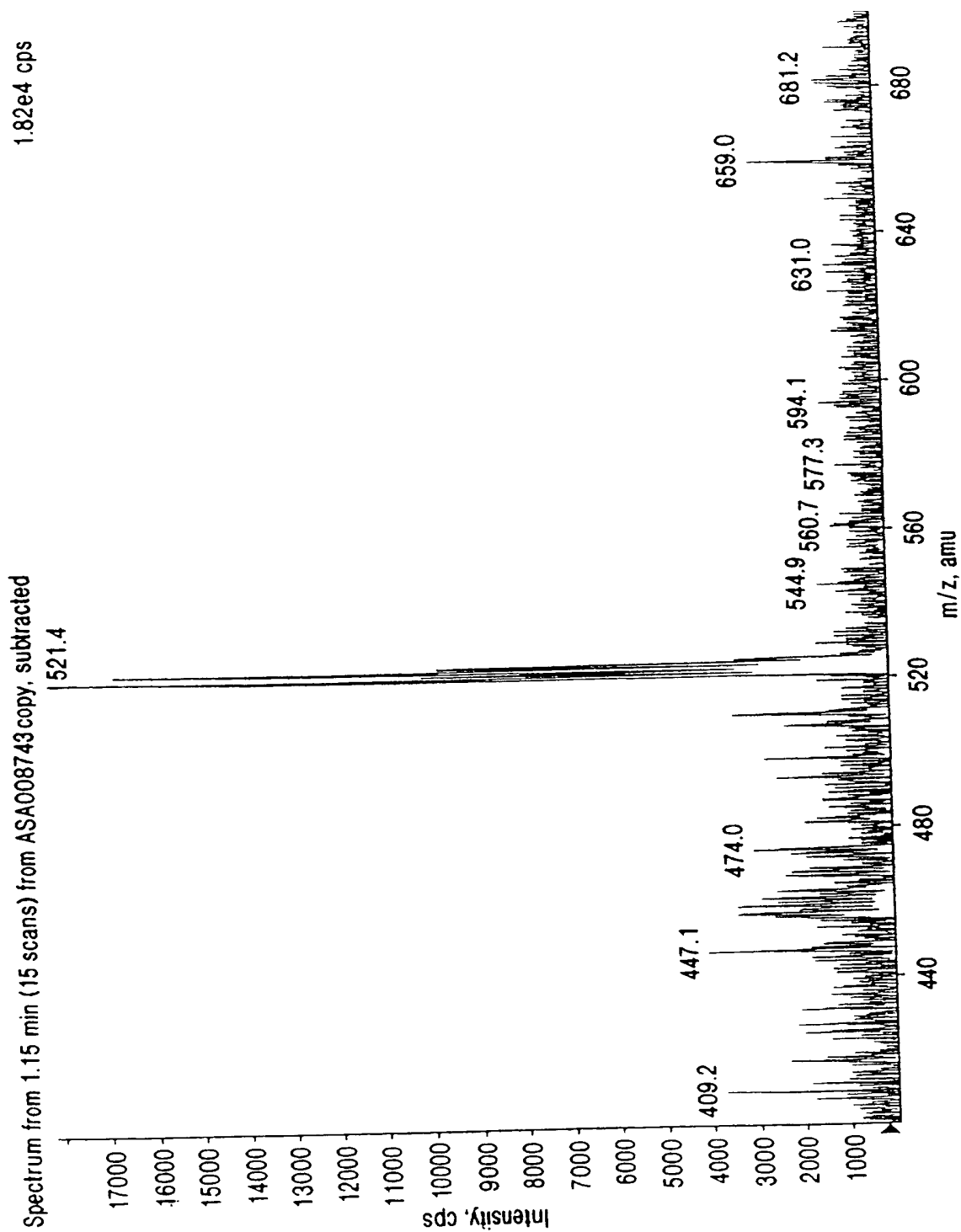


FIG. 203

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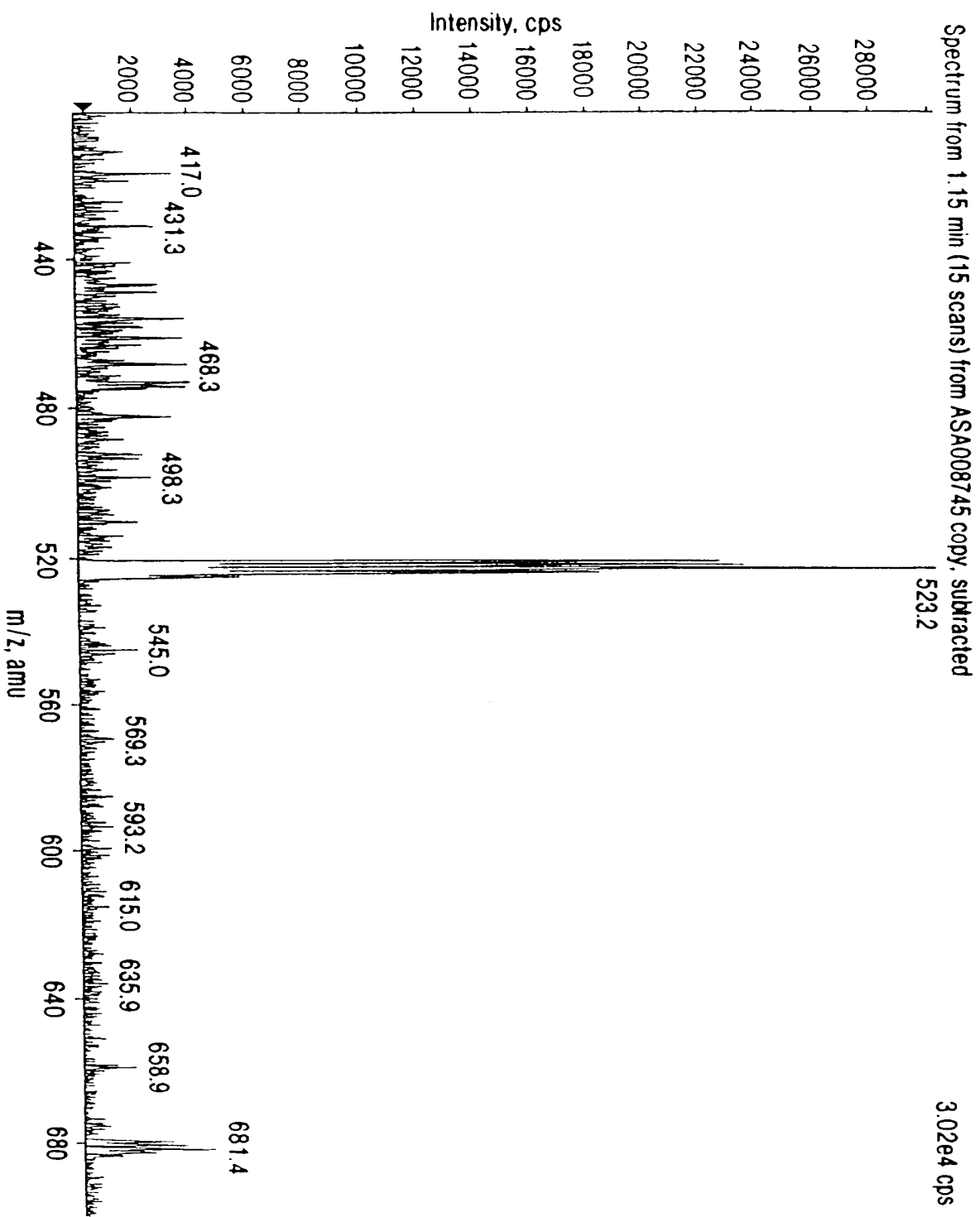


FIG. 204

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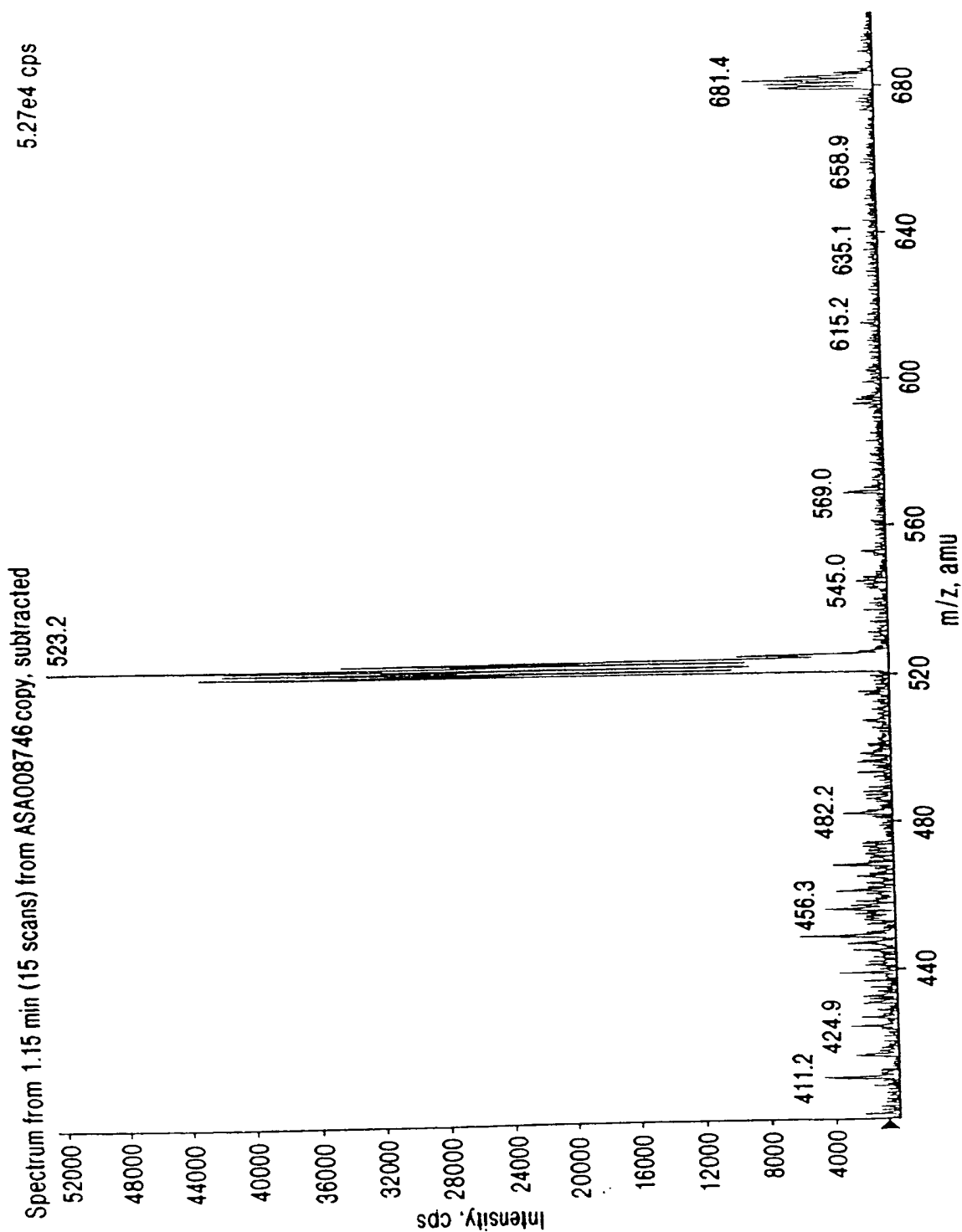


FIG. 205

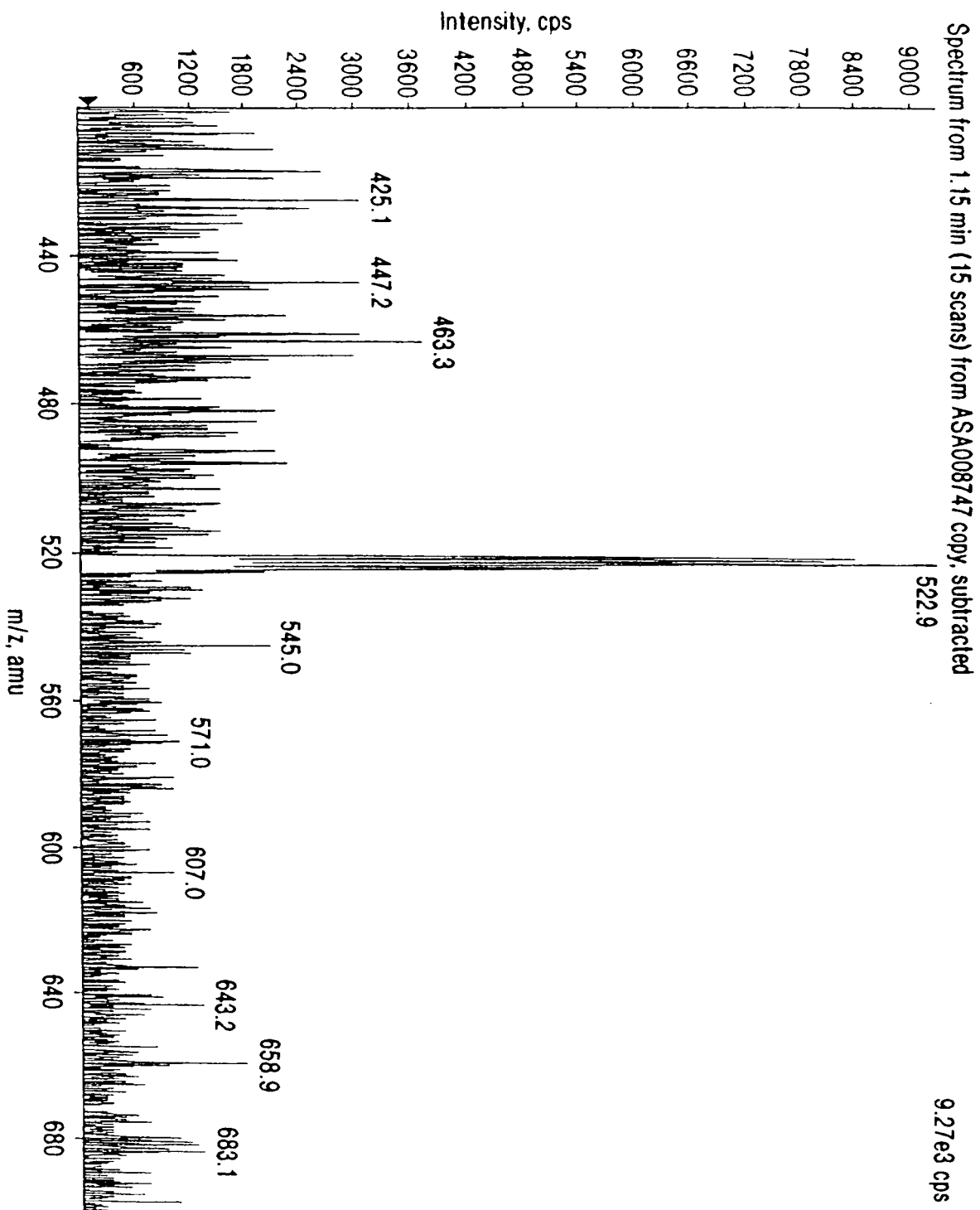


FIG. 206

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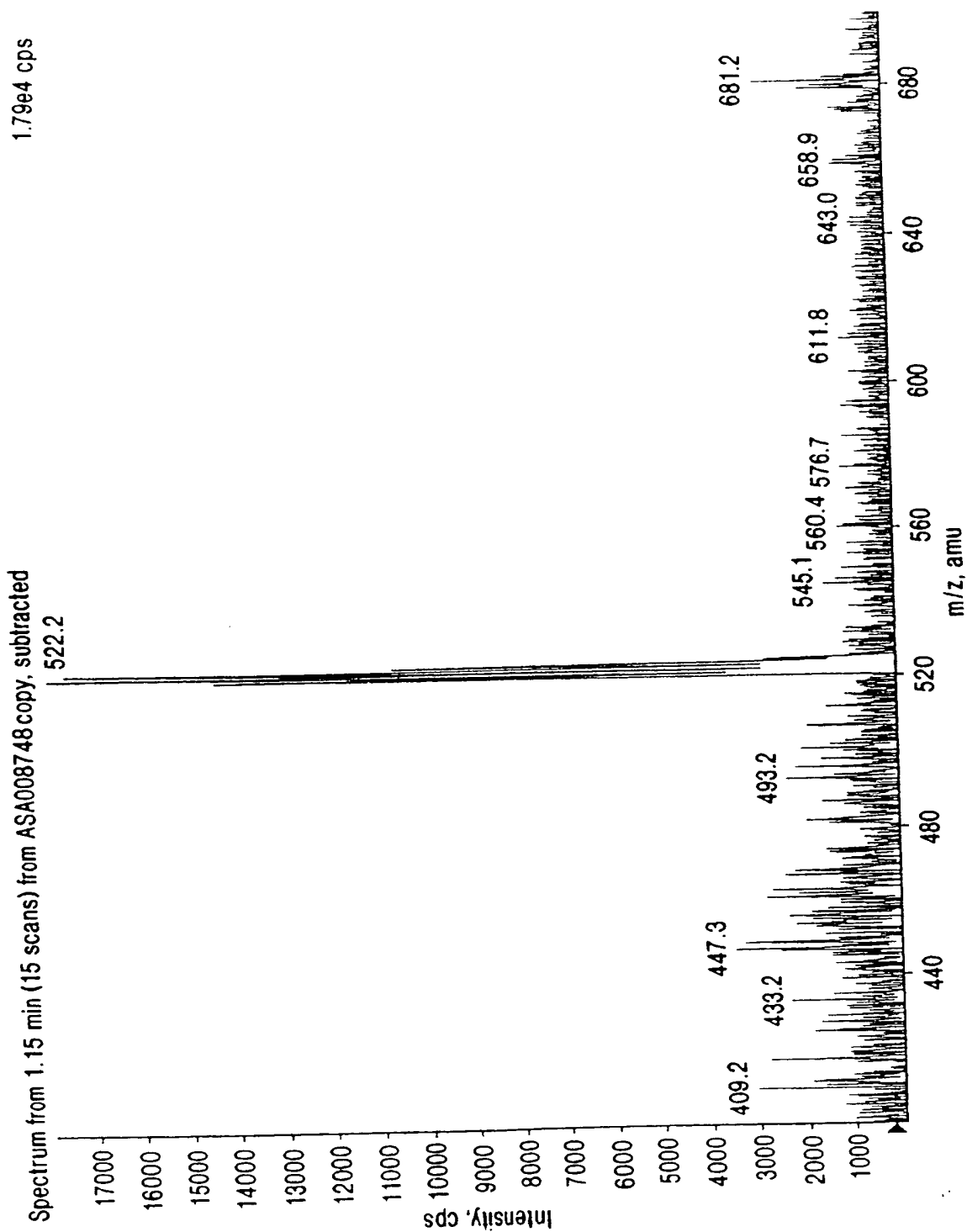


FIG. 207

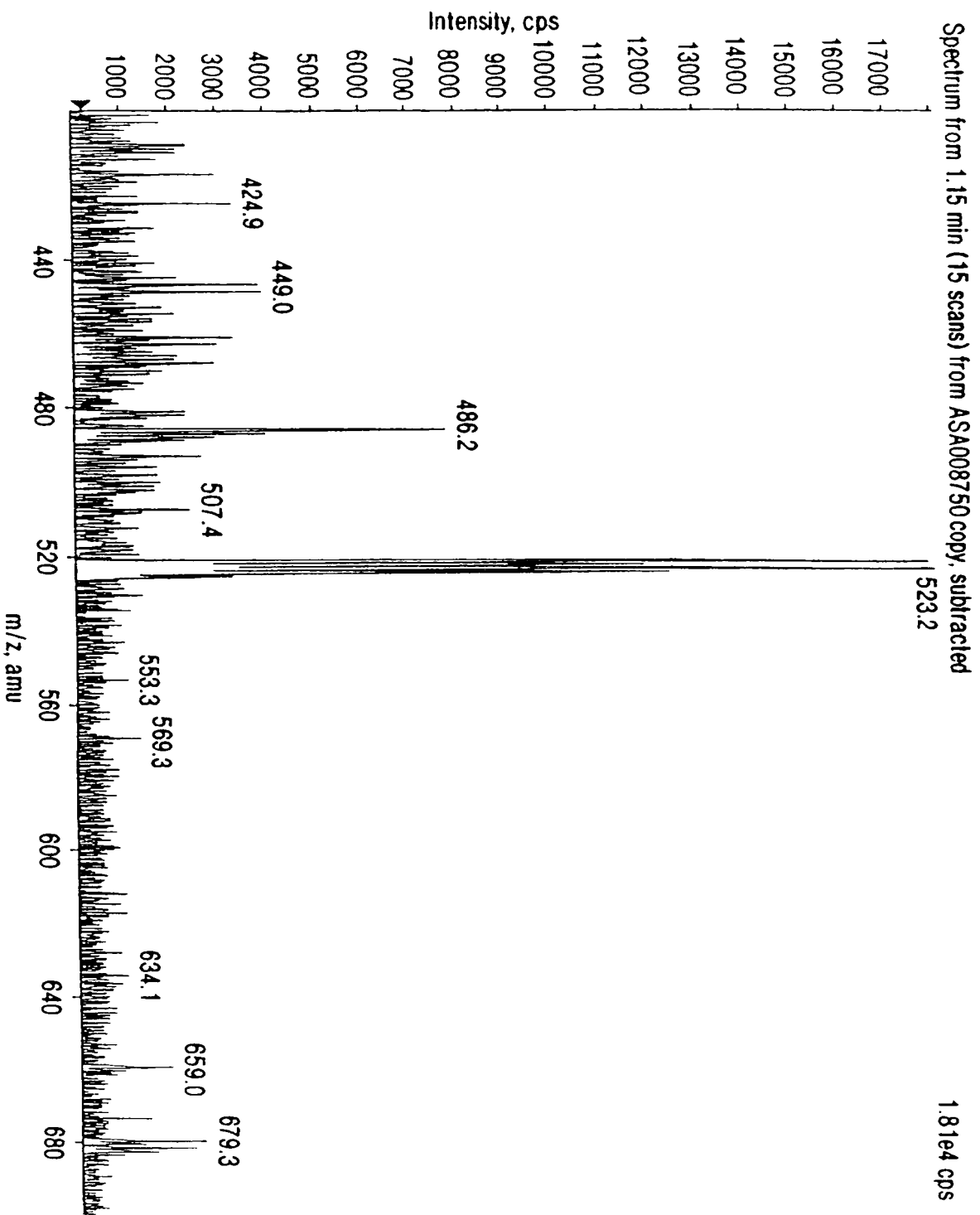


FIG. 208

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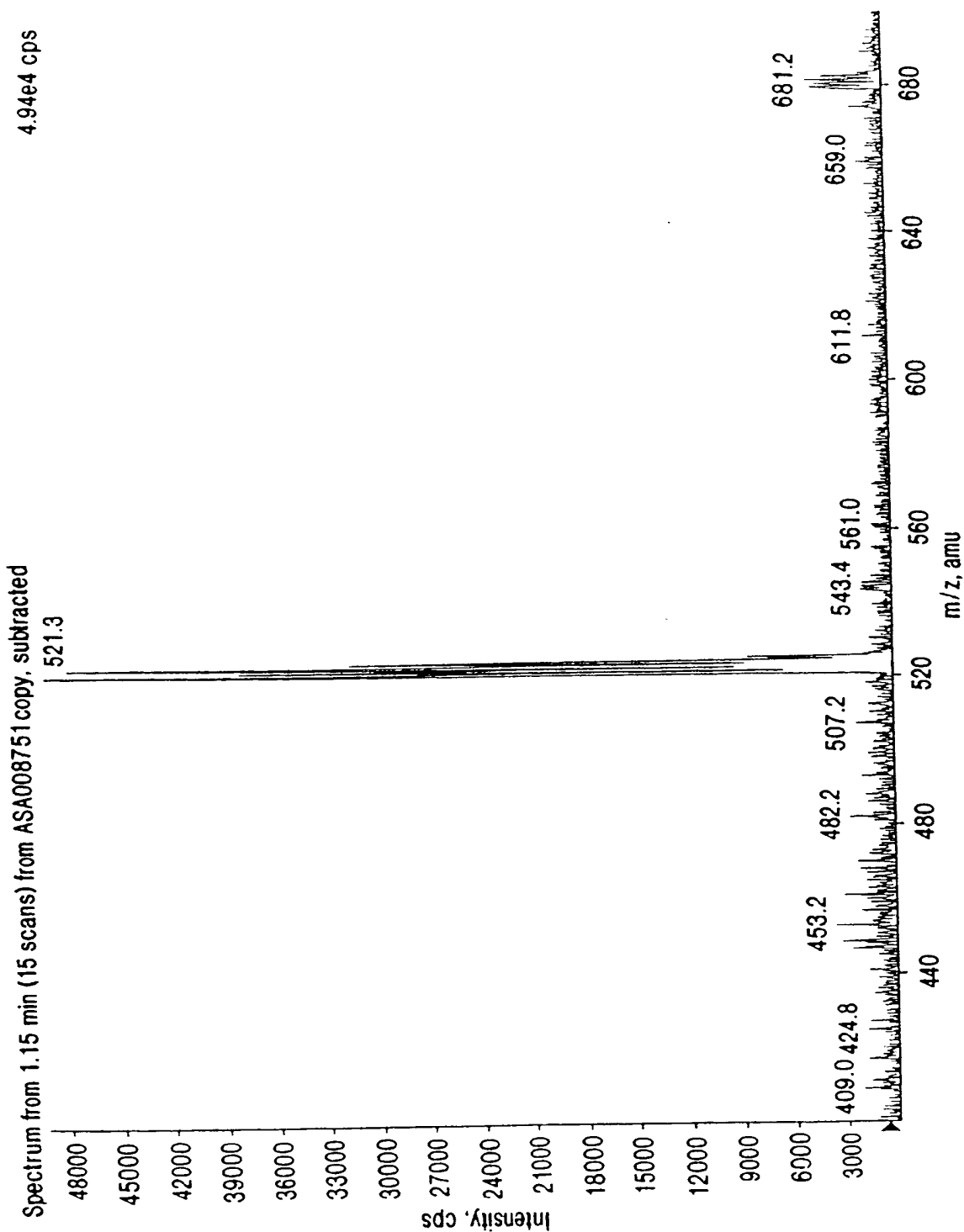


FIG. 209

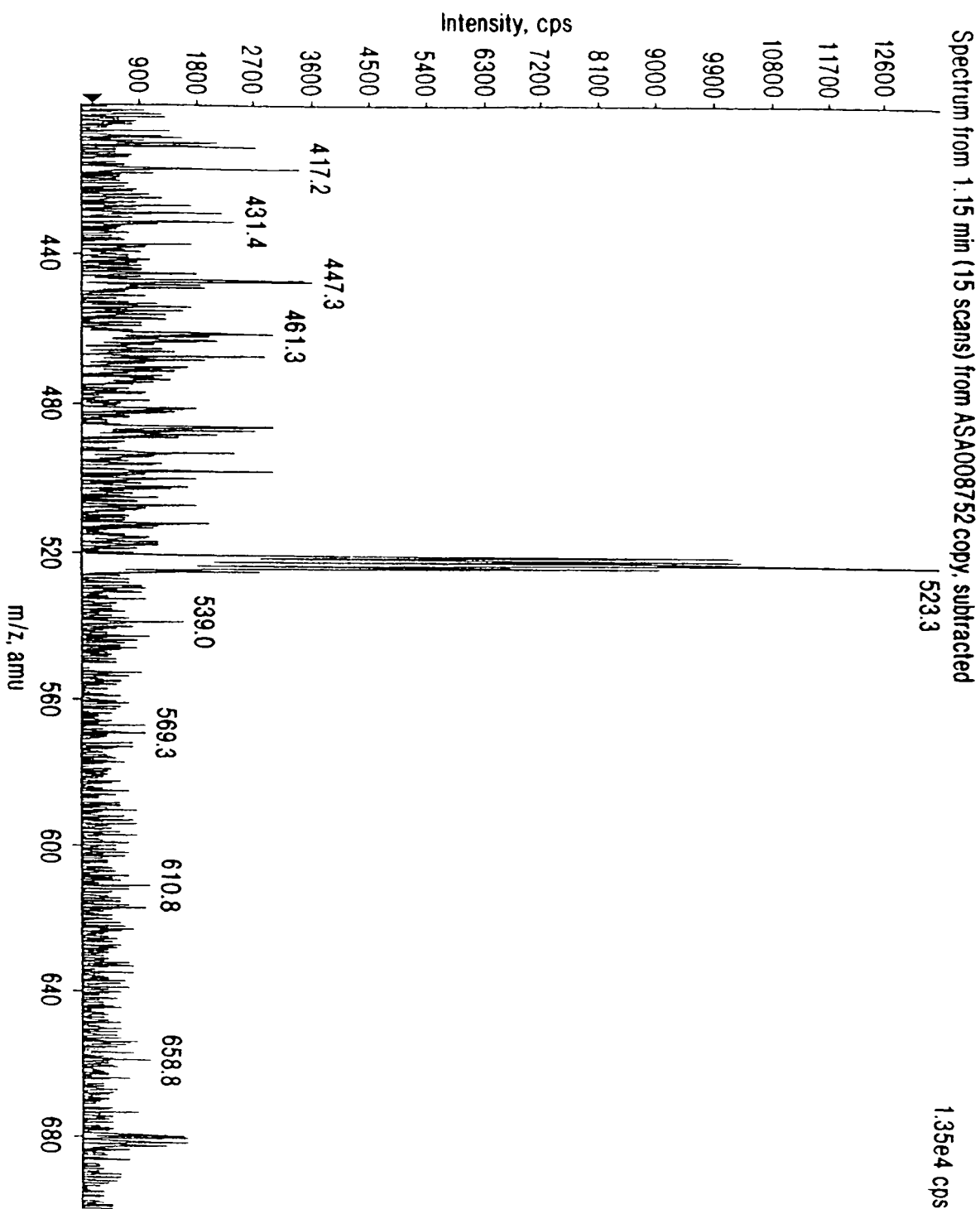


FIG. 210

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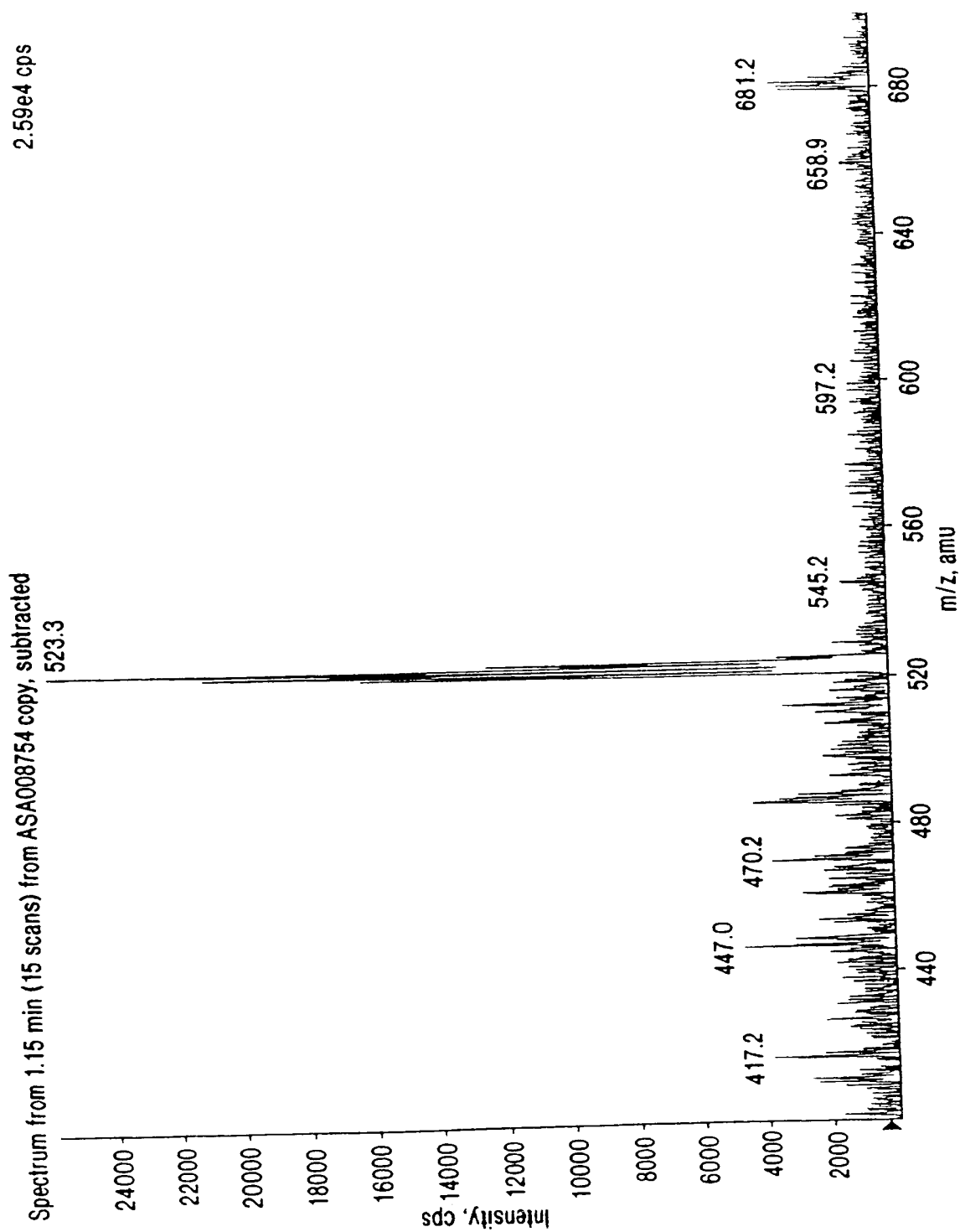


FIG. 211

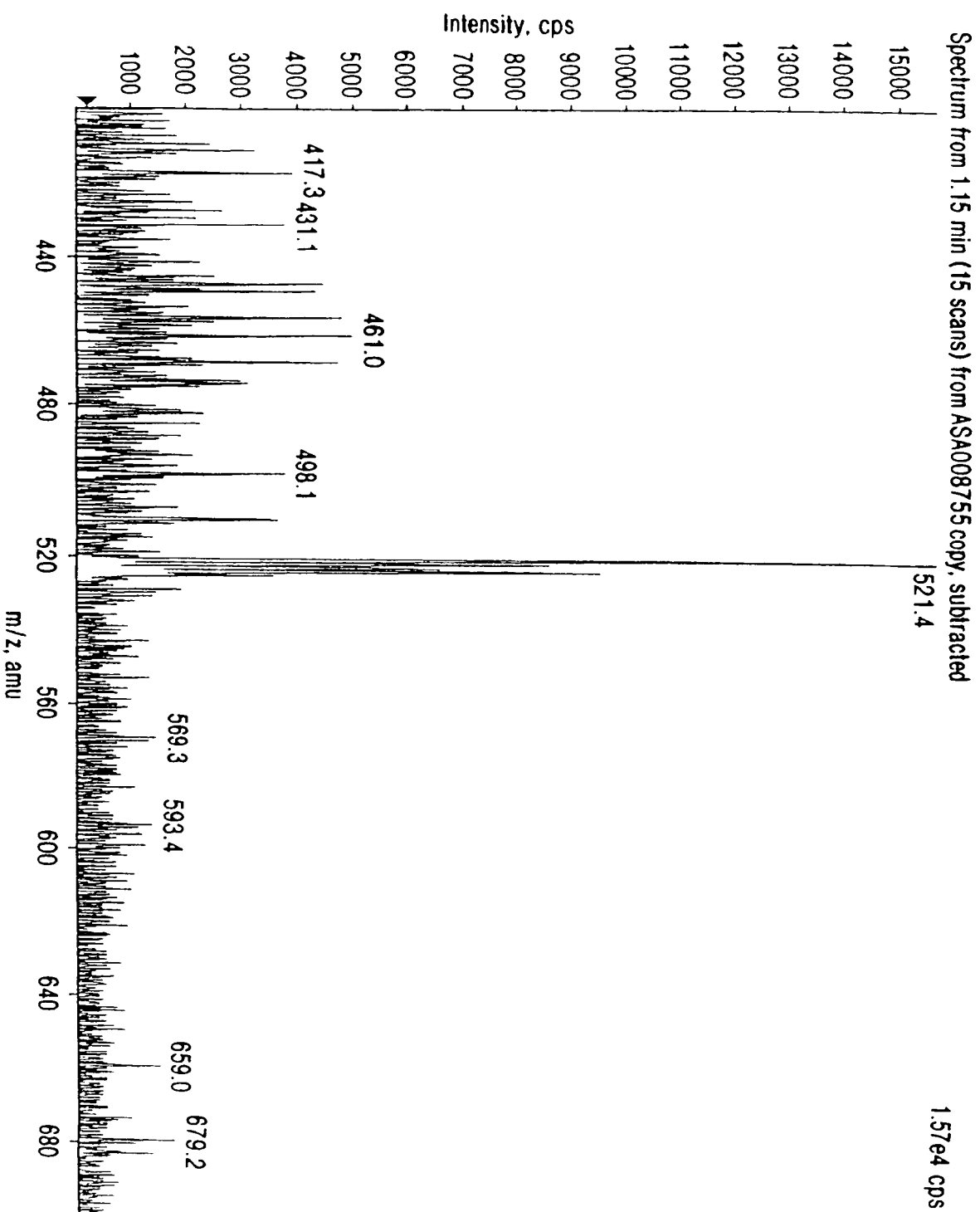


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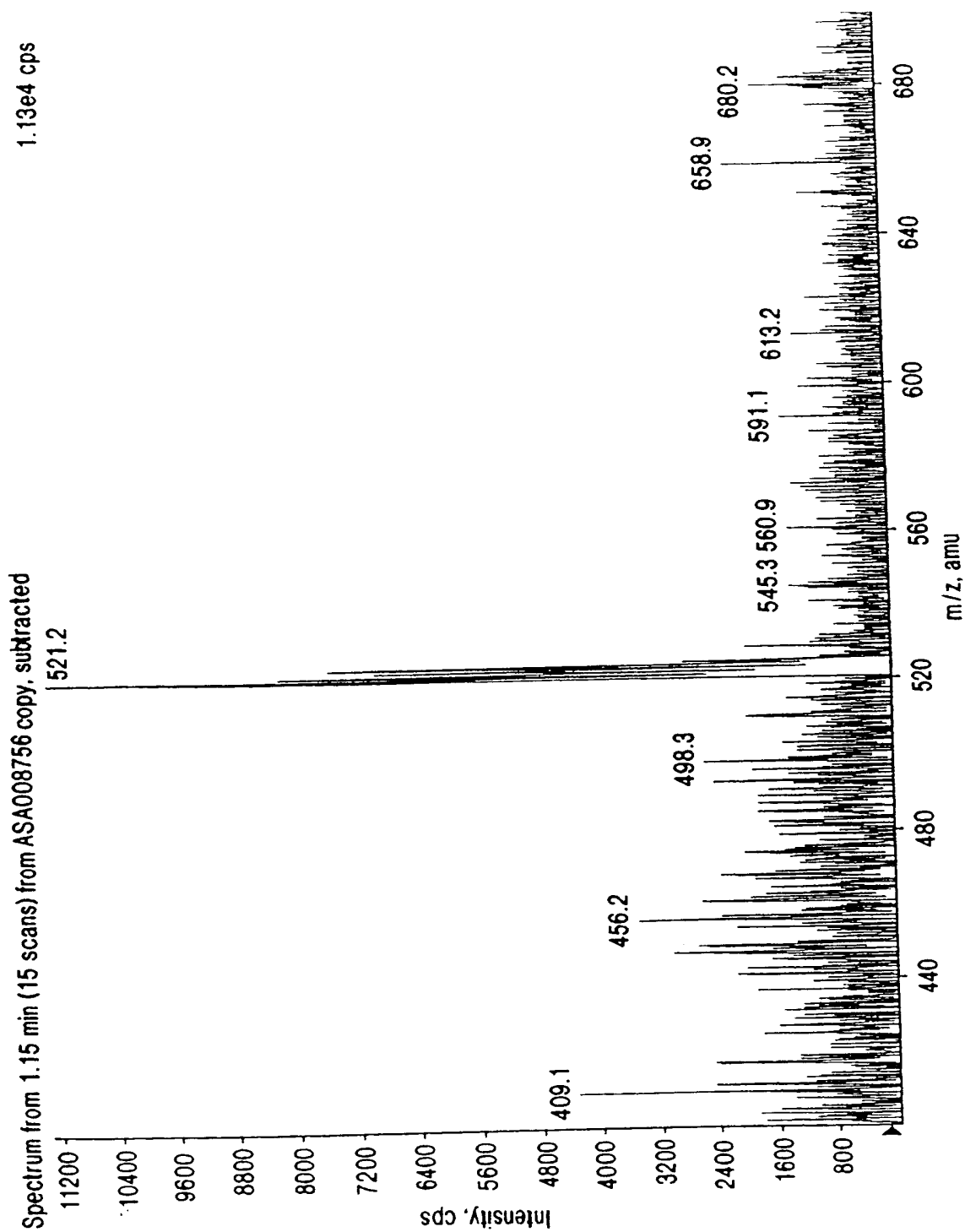


FIG. 213

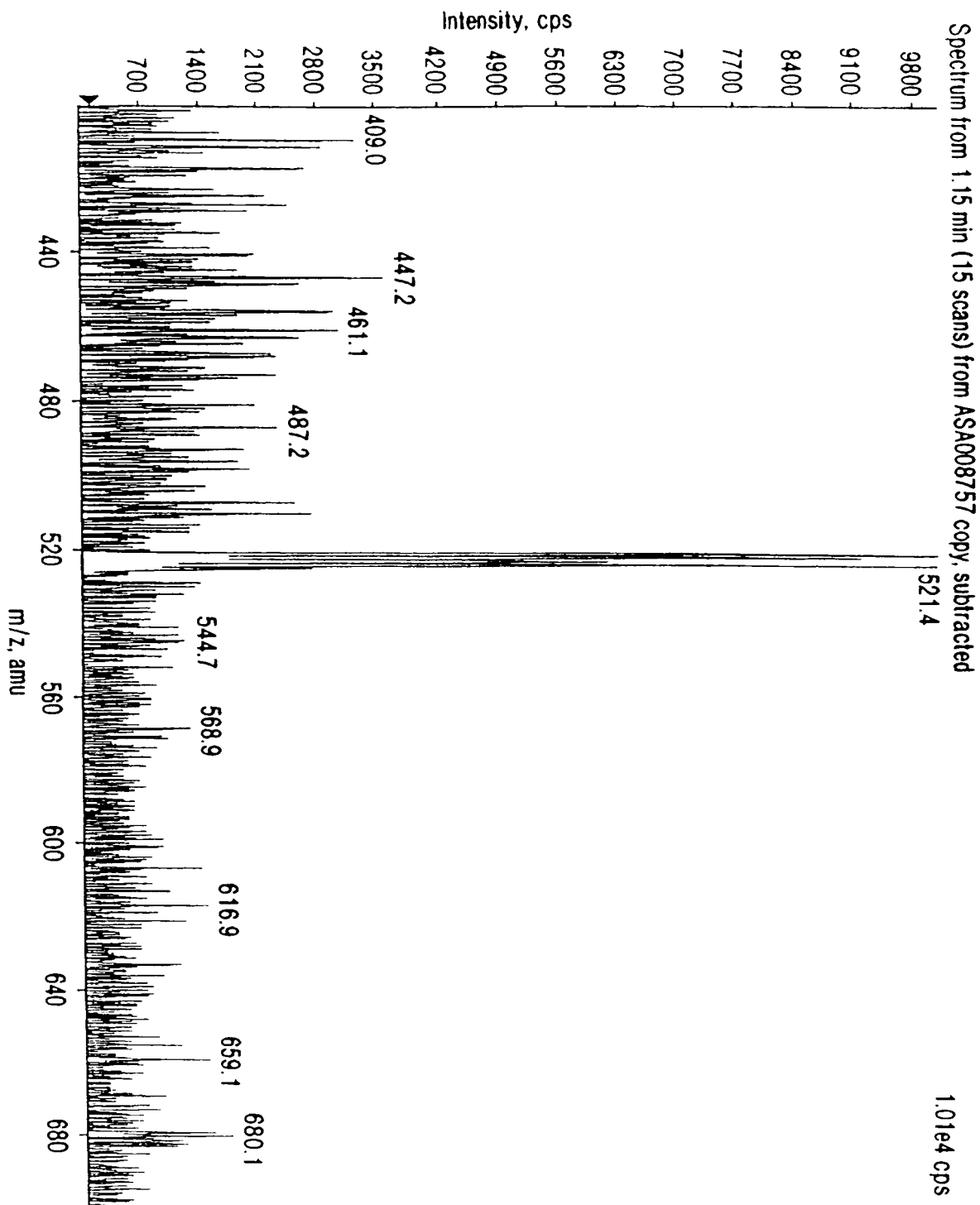


FIG. 214

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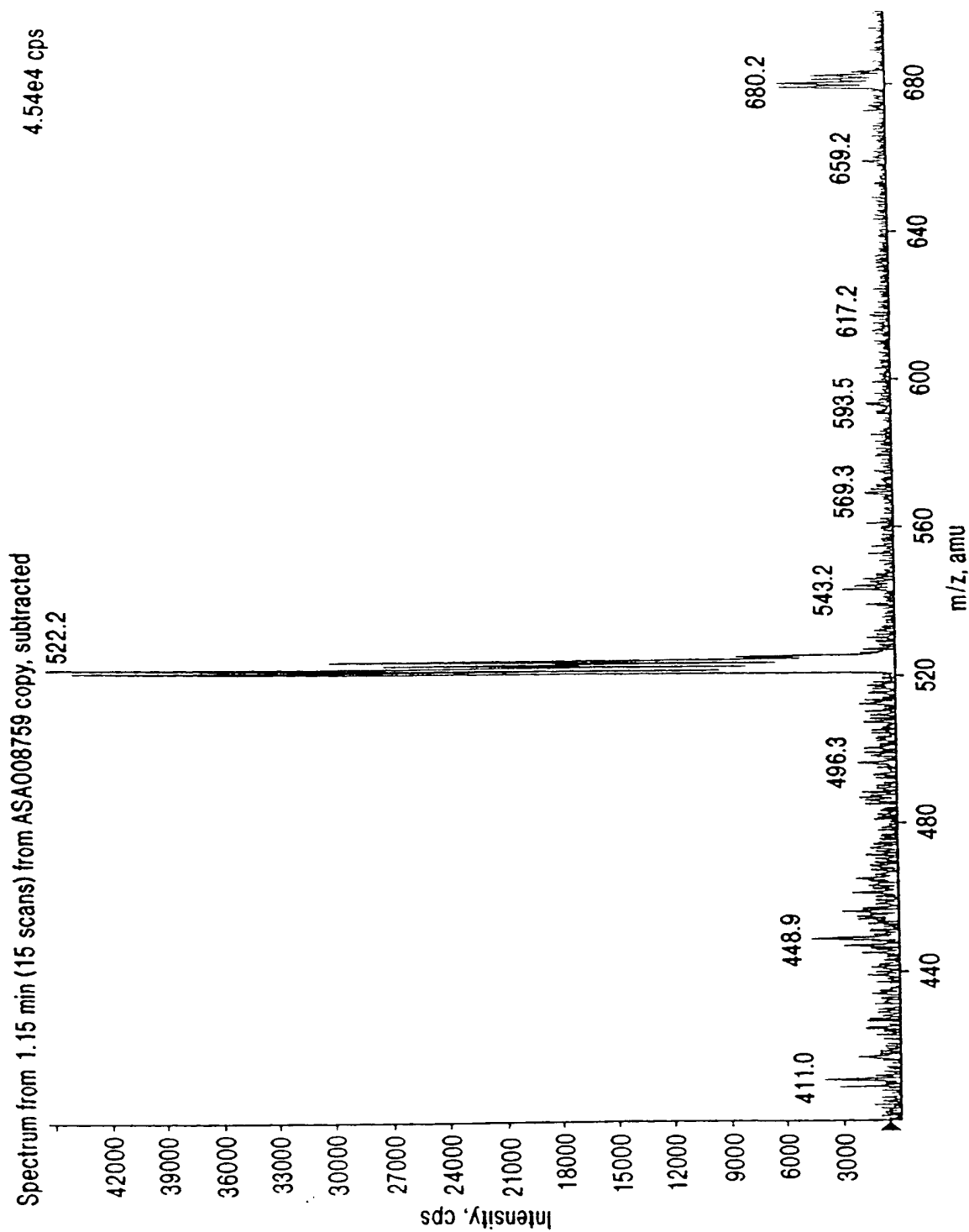


FIG. 215

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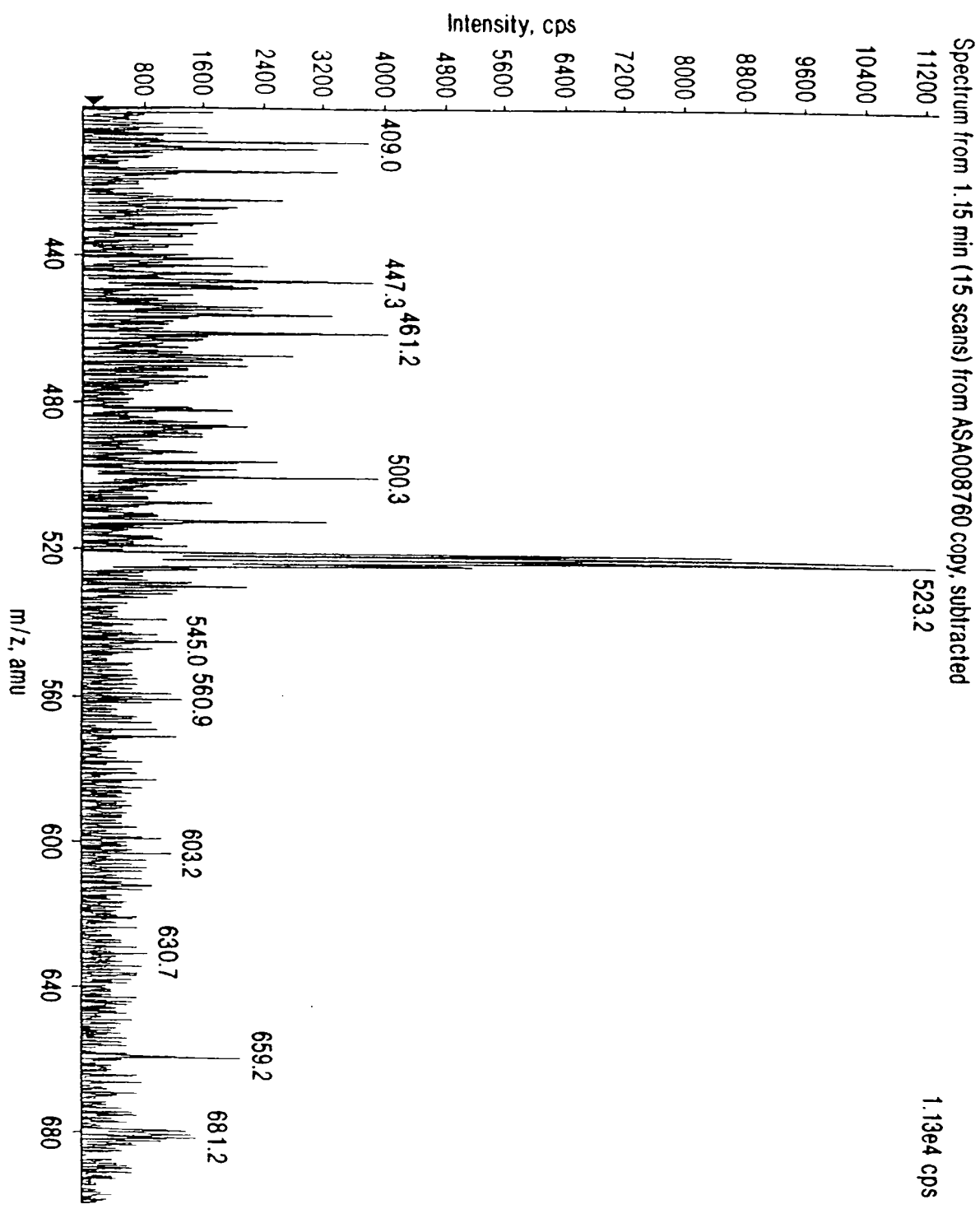


FIG. 216

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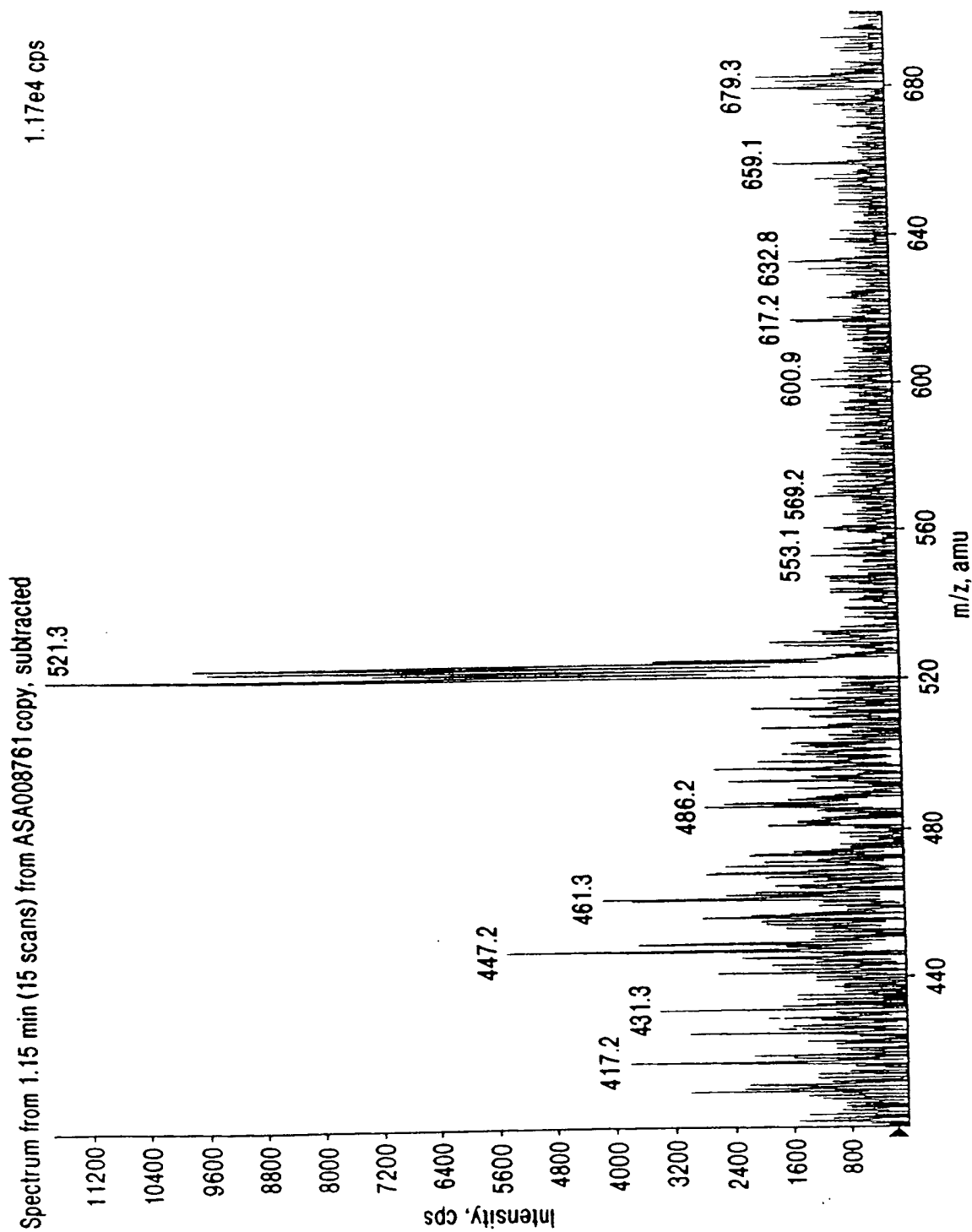


FIG. 217

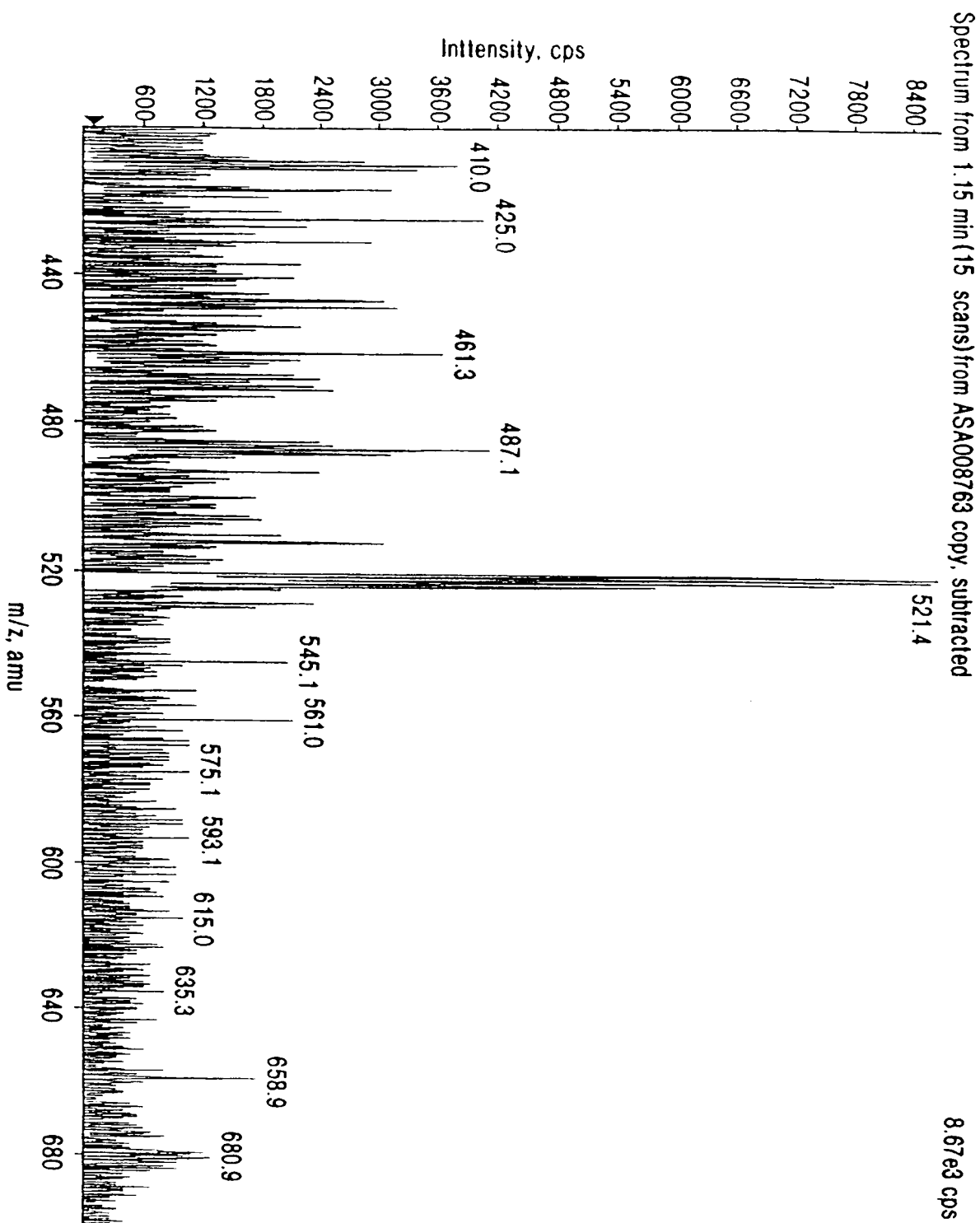


FIG. 218

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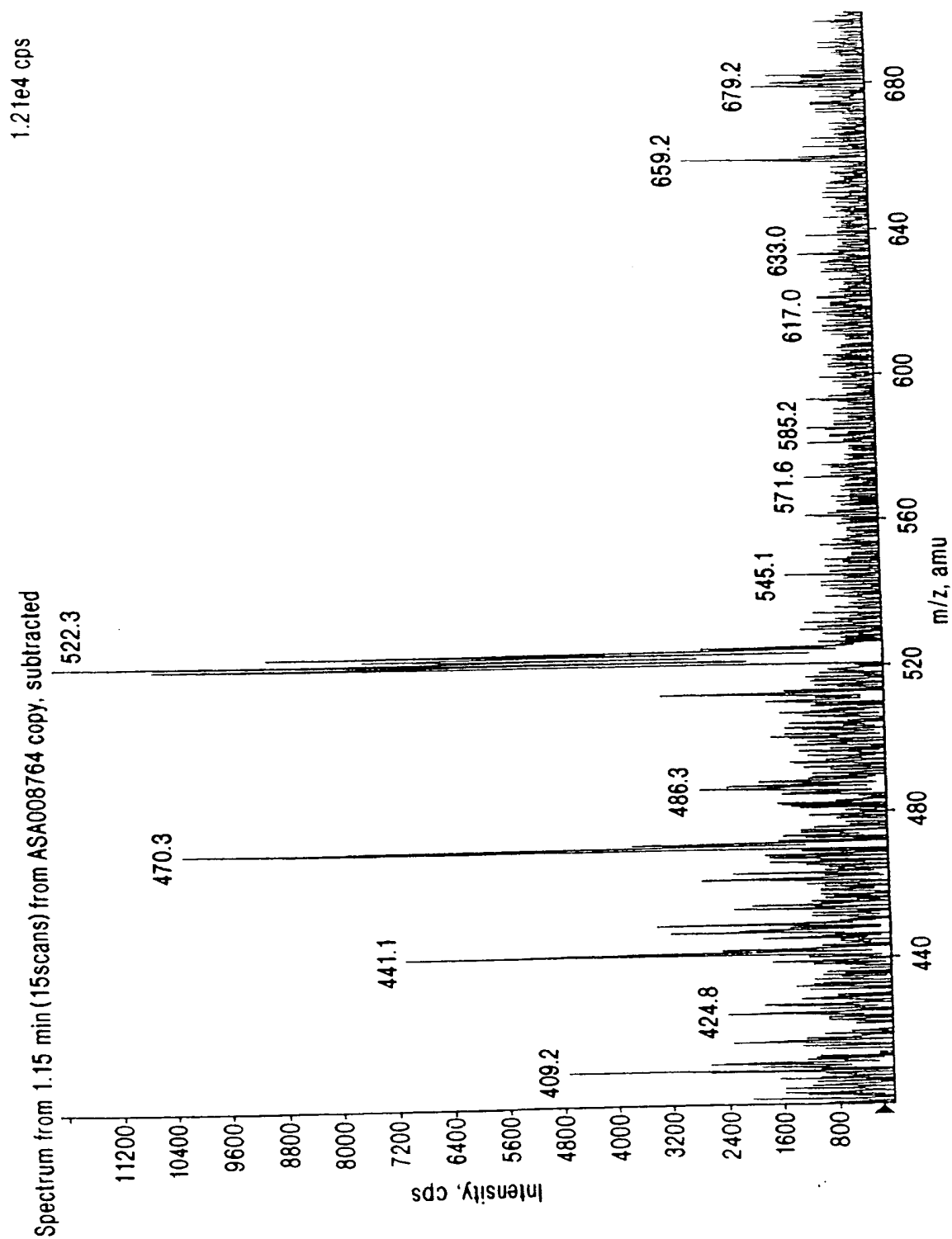


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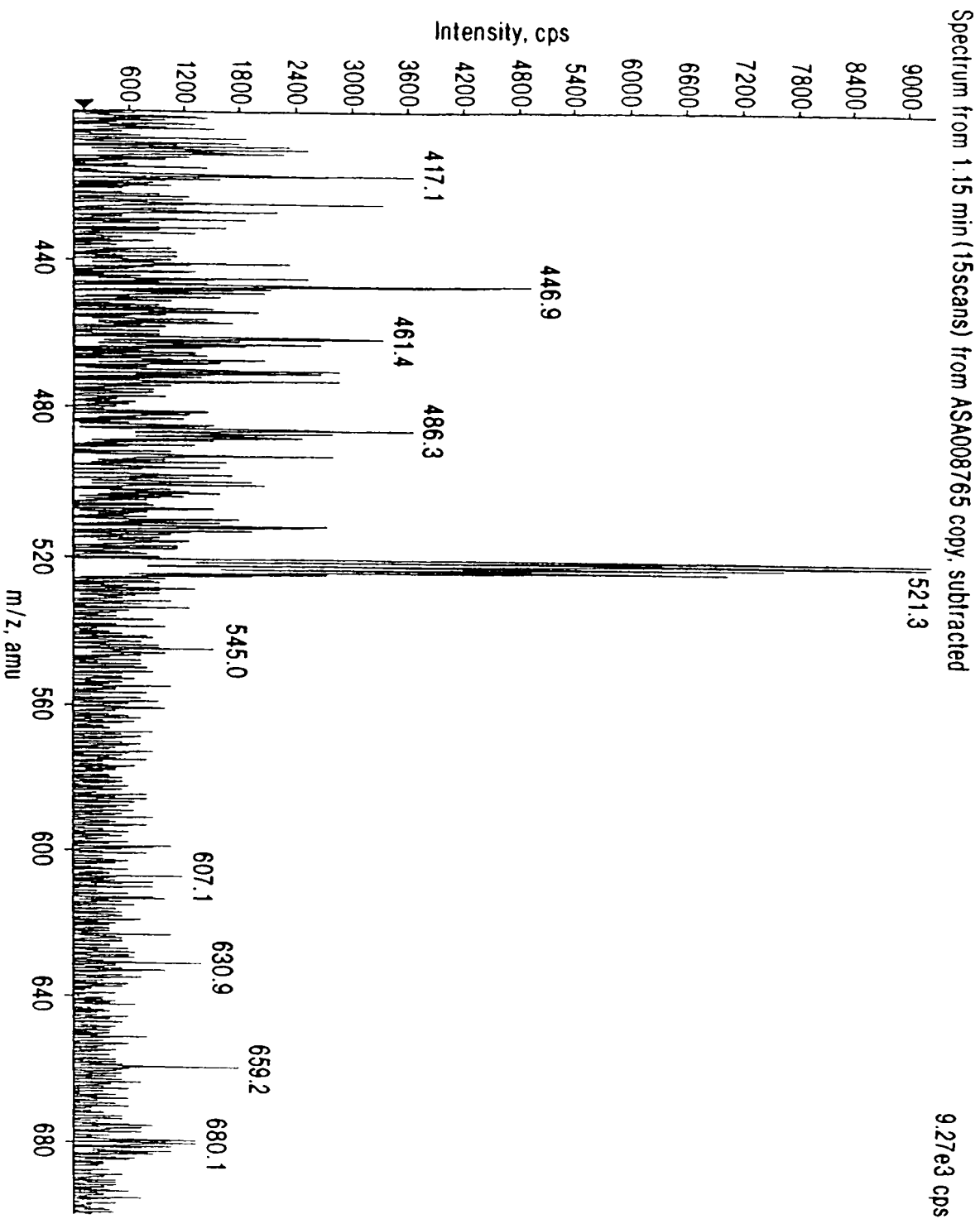


FIG. 220

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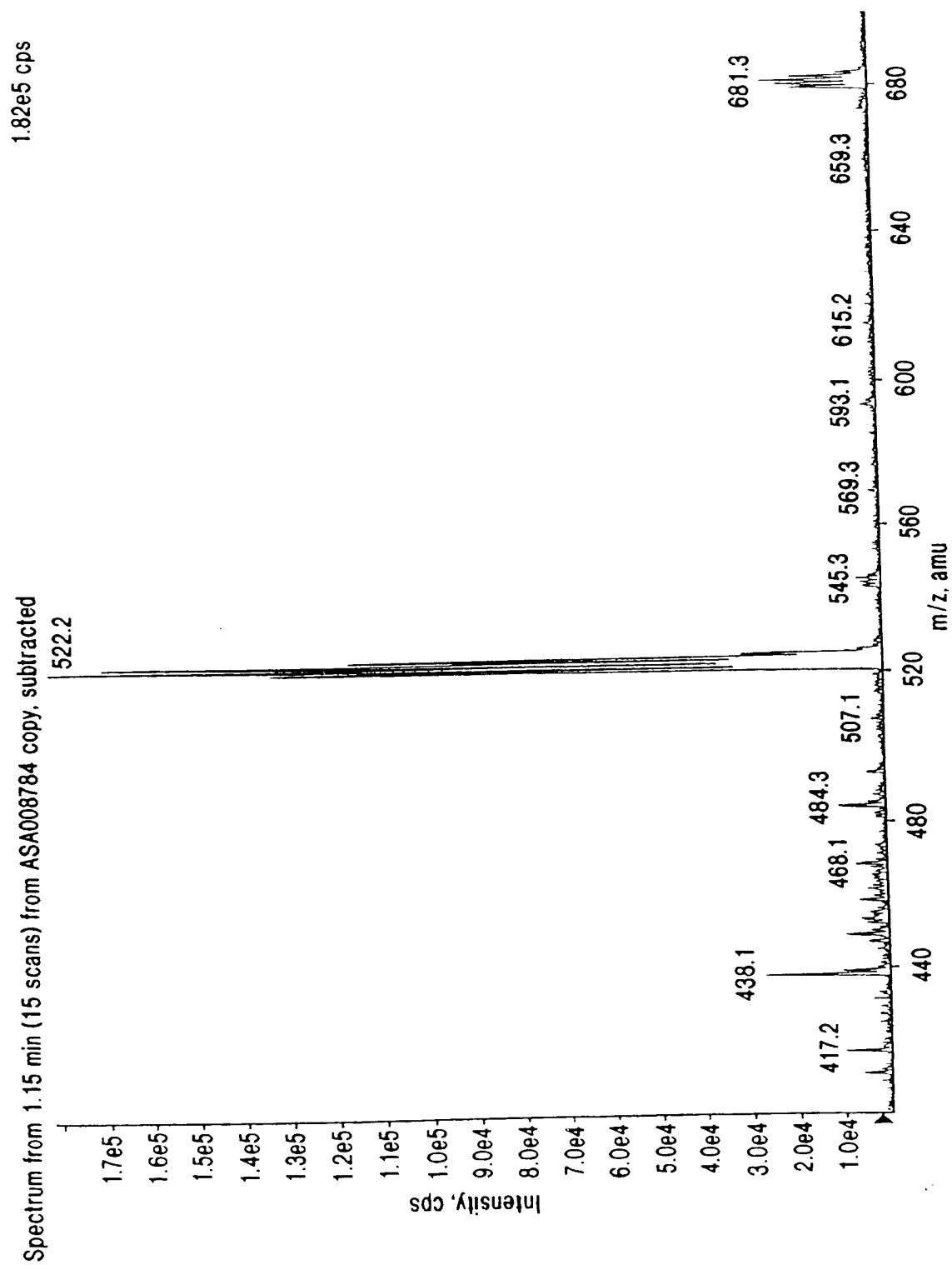


FIG. 221

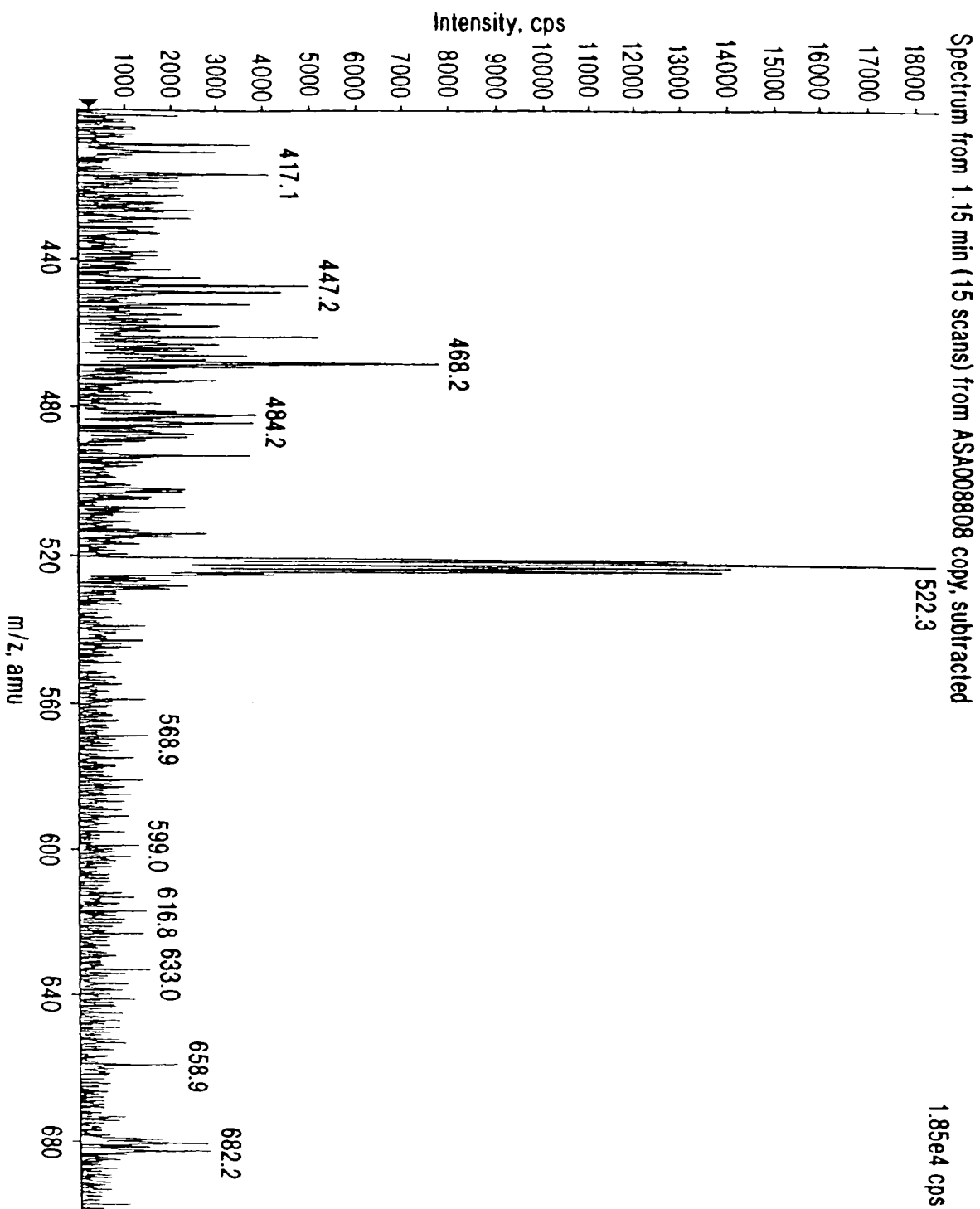


FIG. 222

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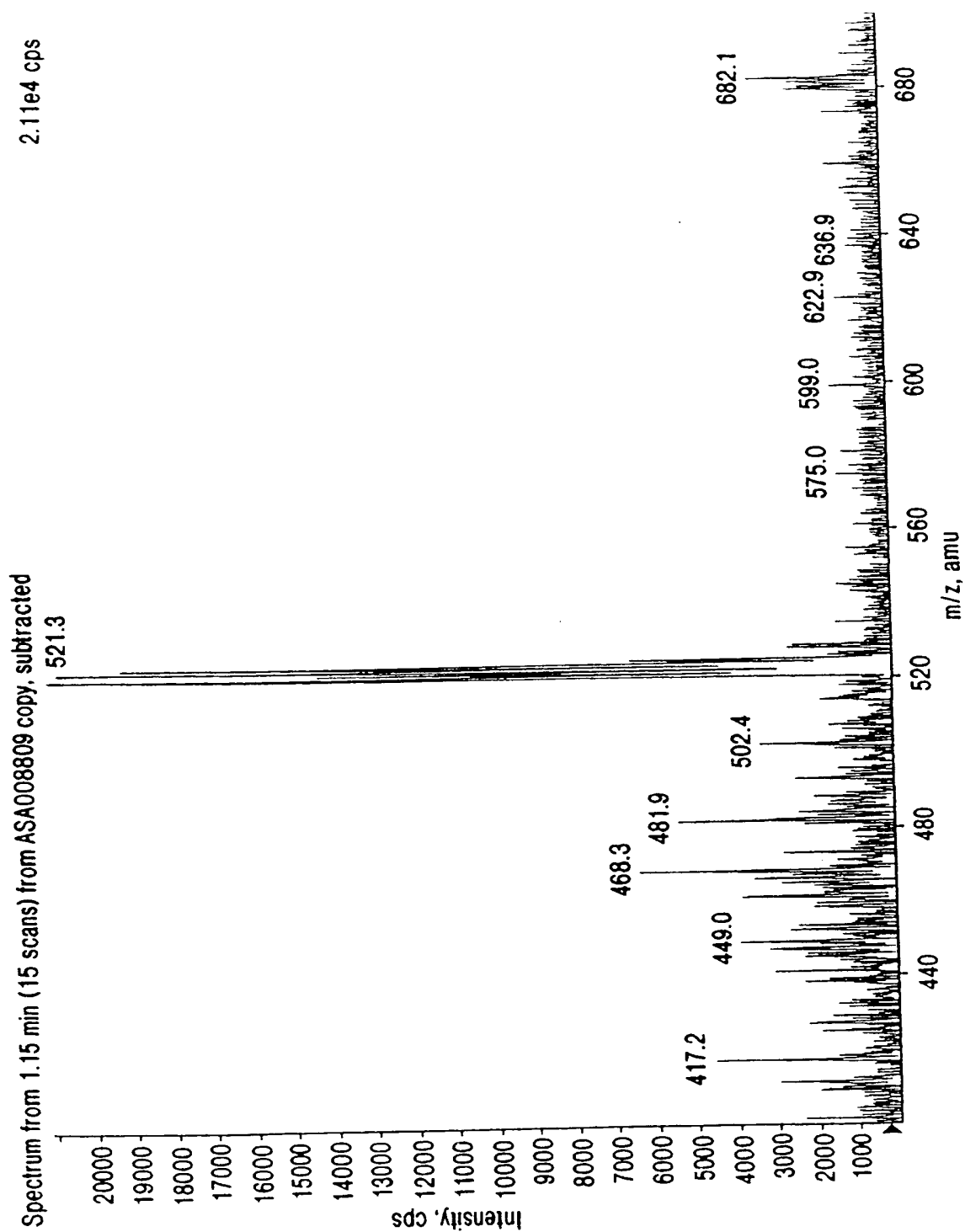


FIG. 223

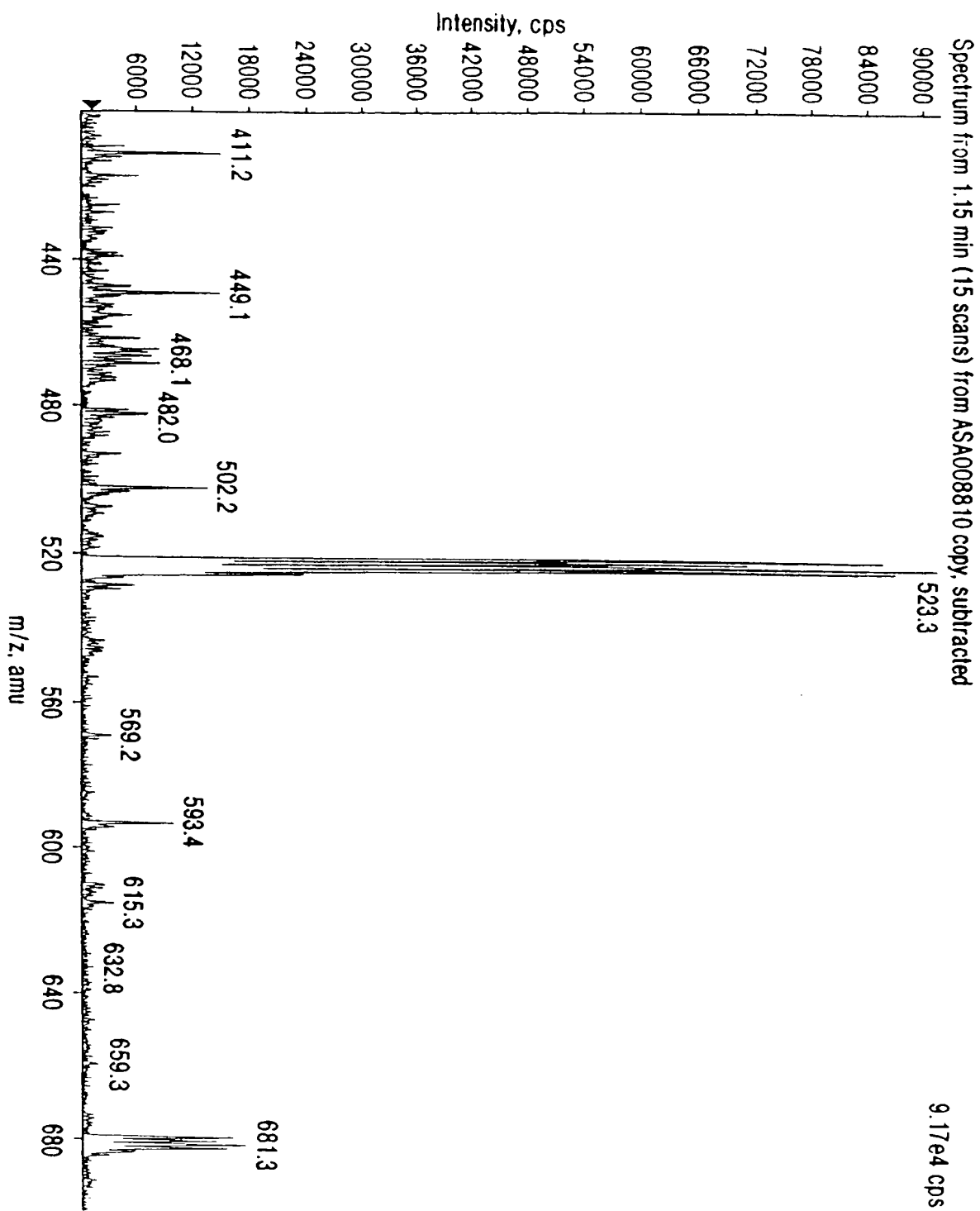


FIG. 224

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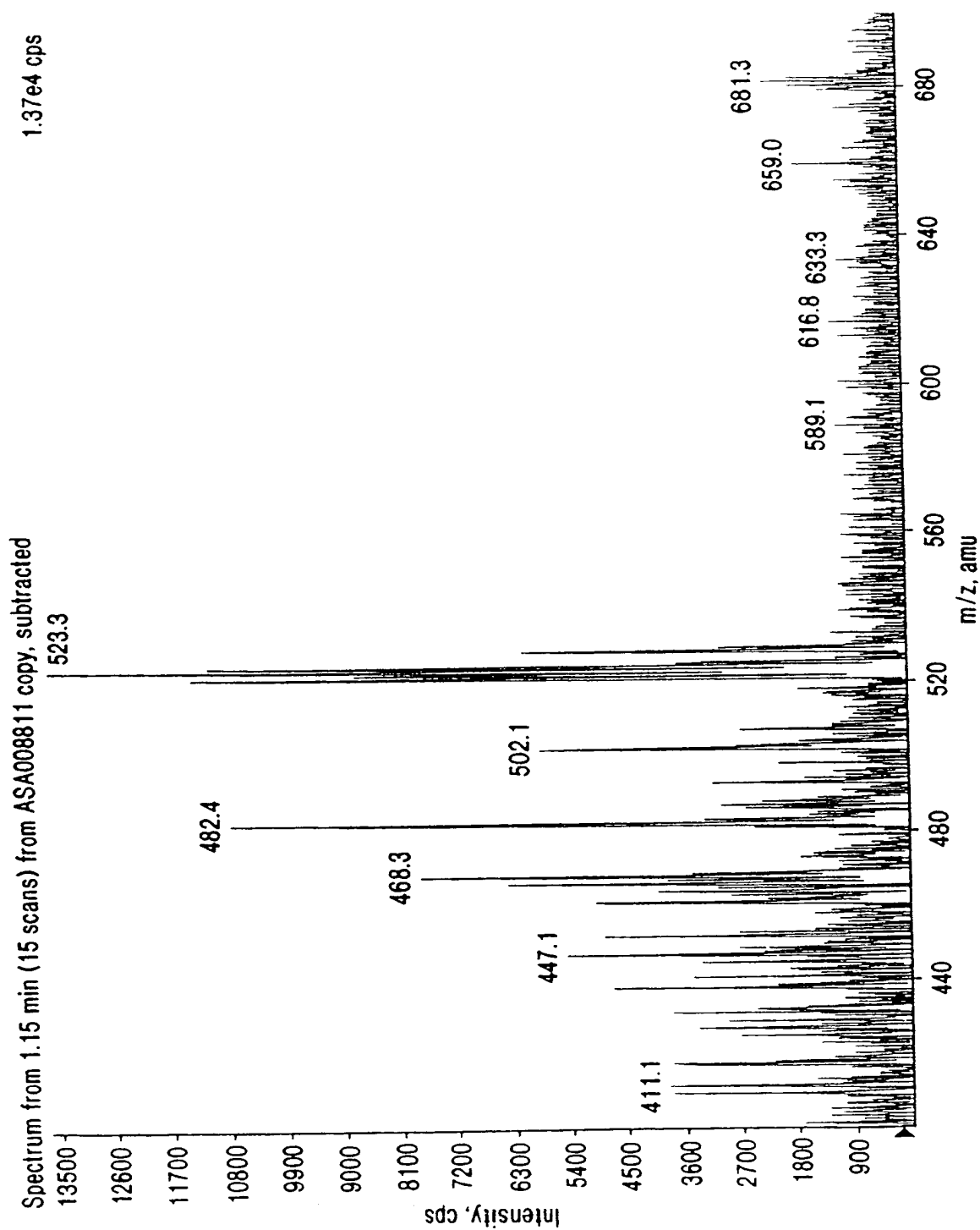


FIG. 225

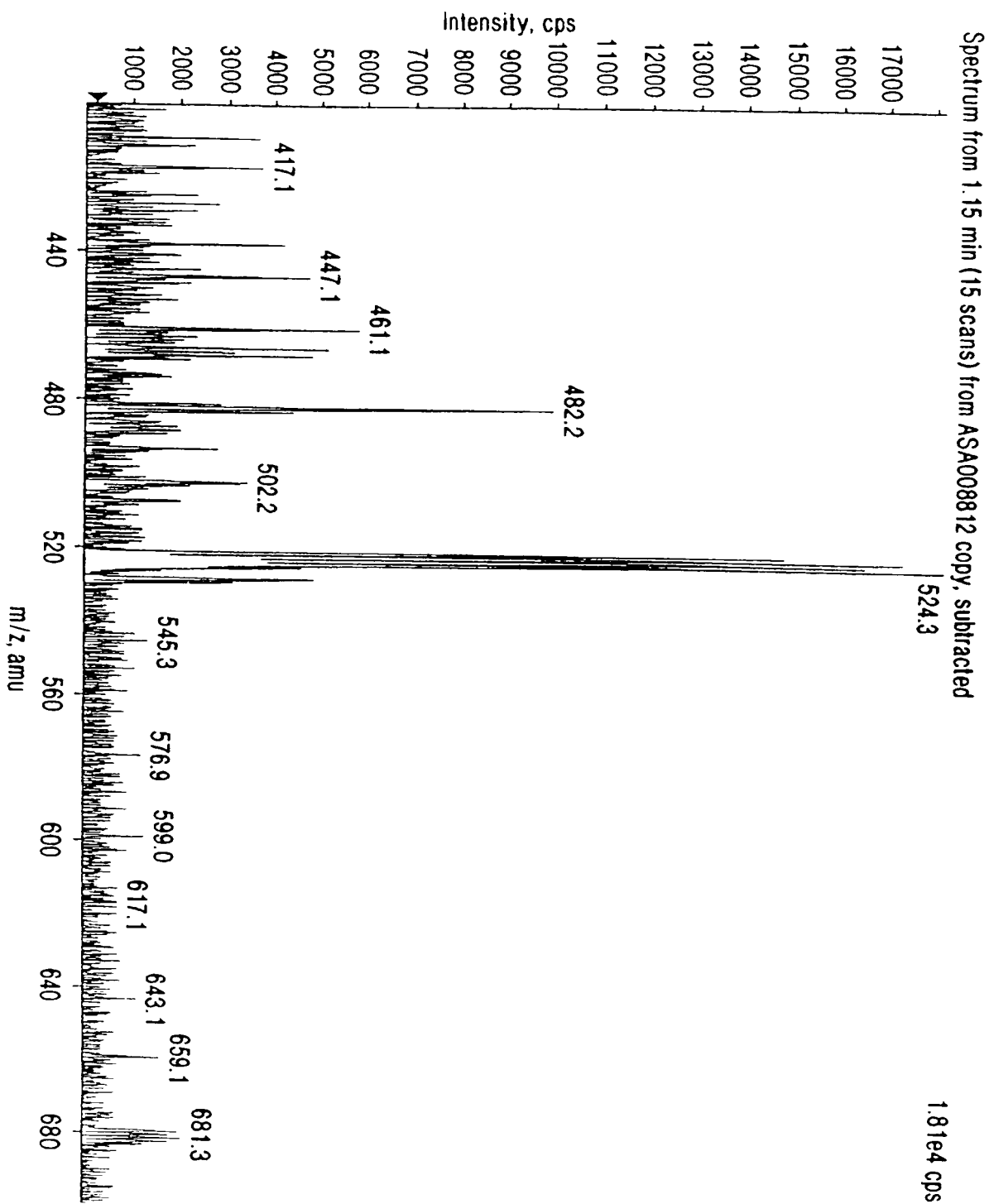


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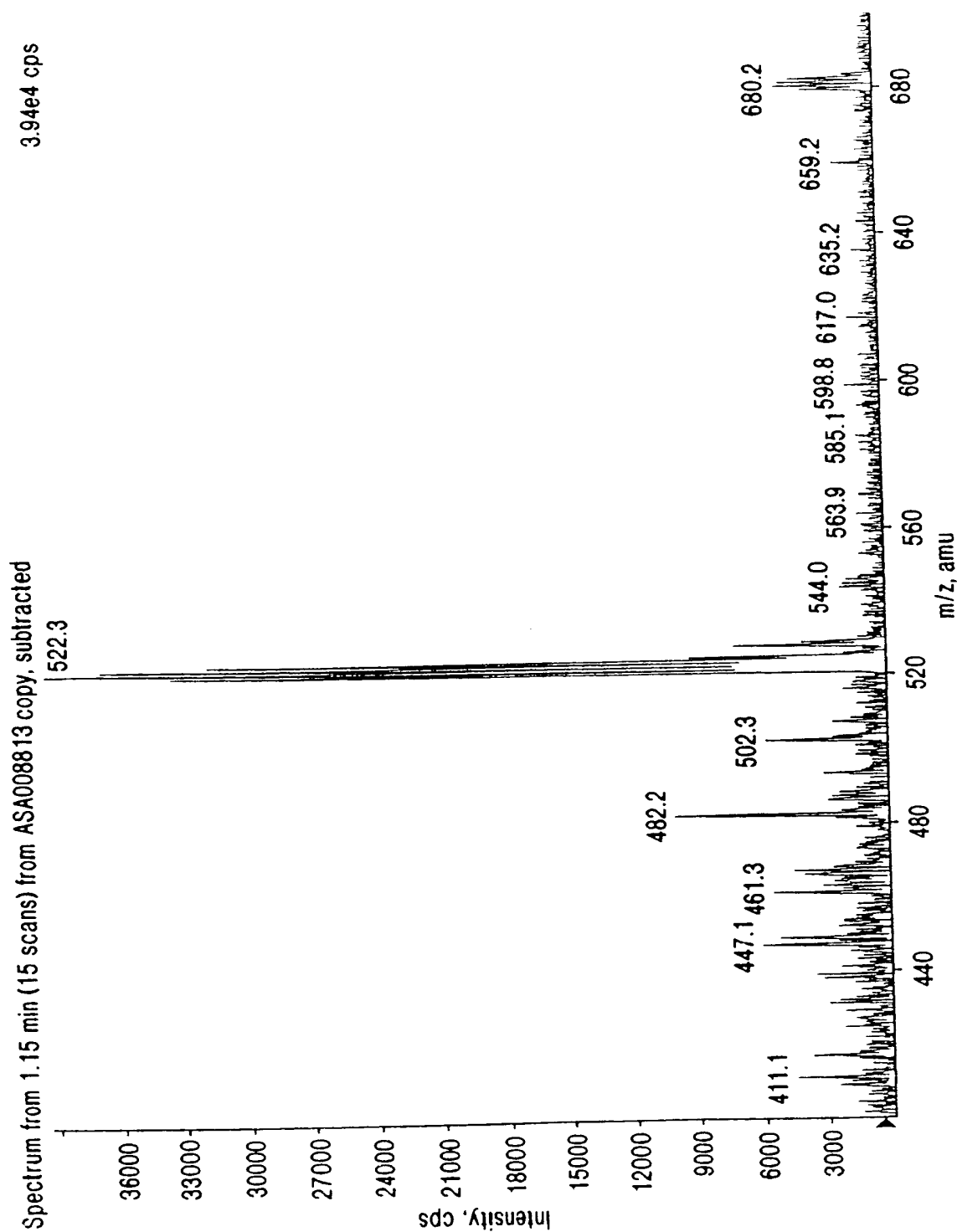


FIG. 227

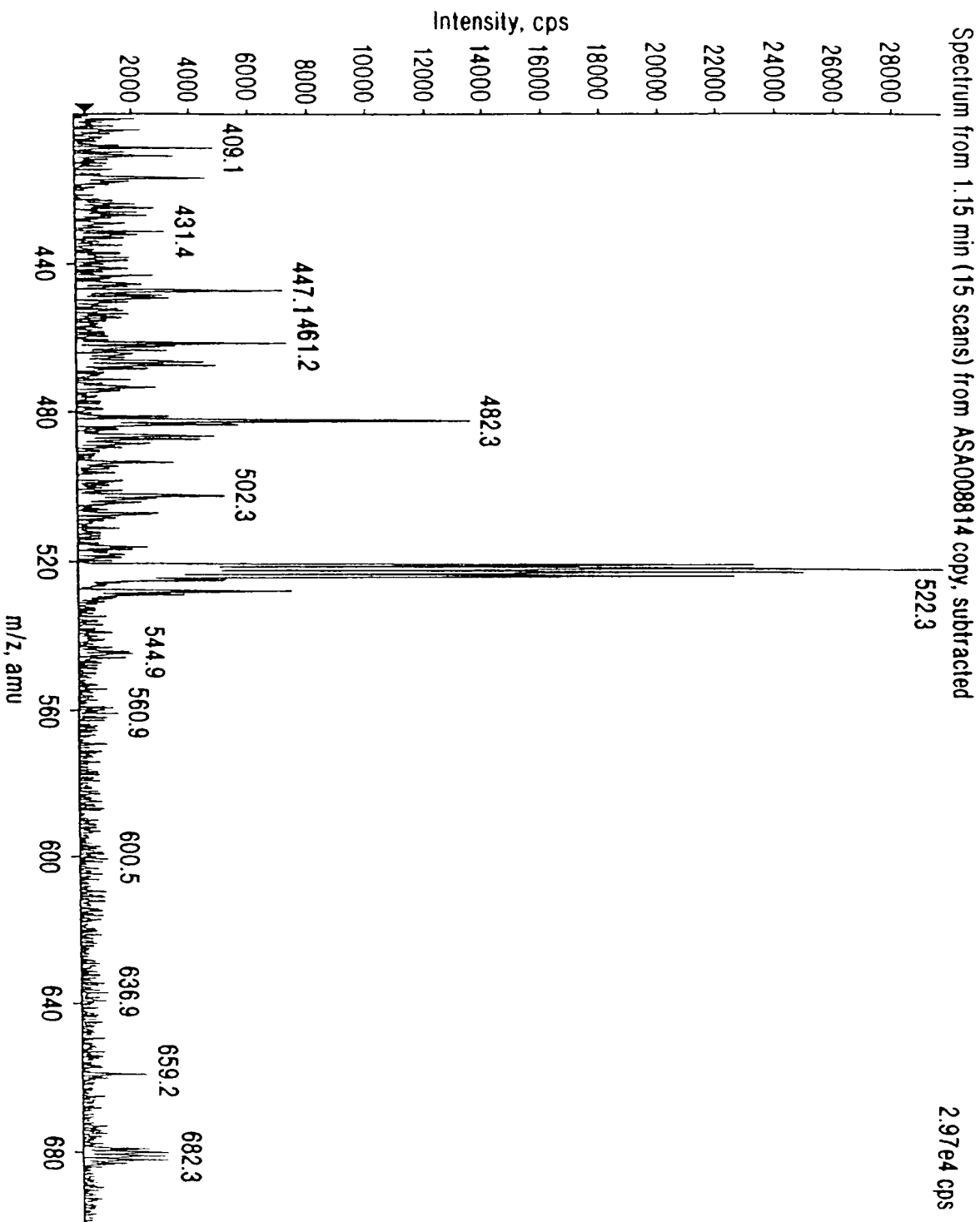


FIG. 228

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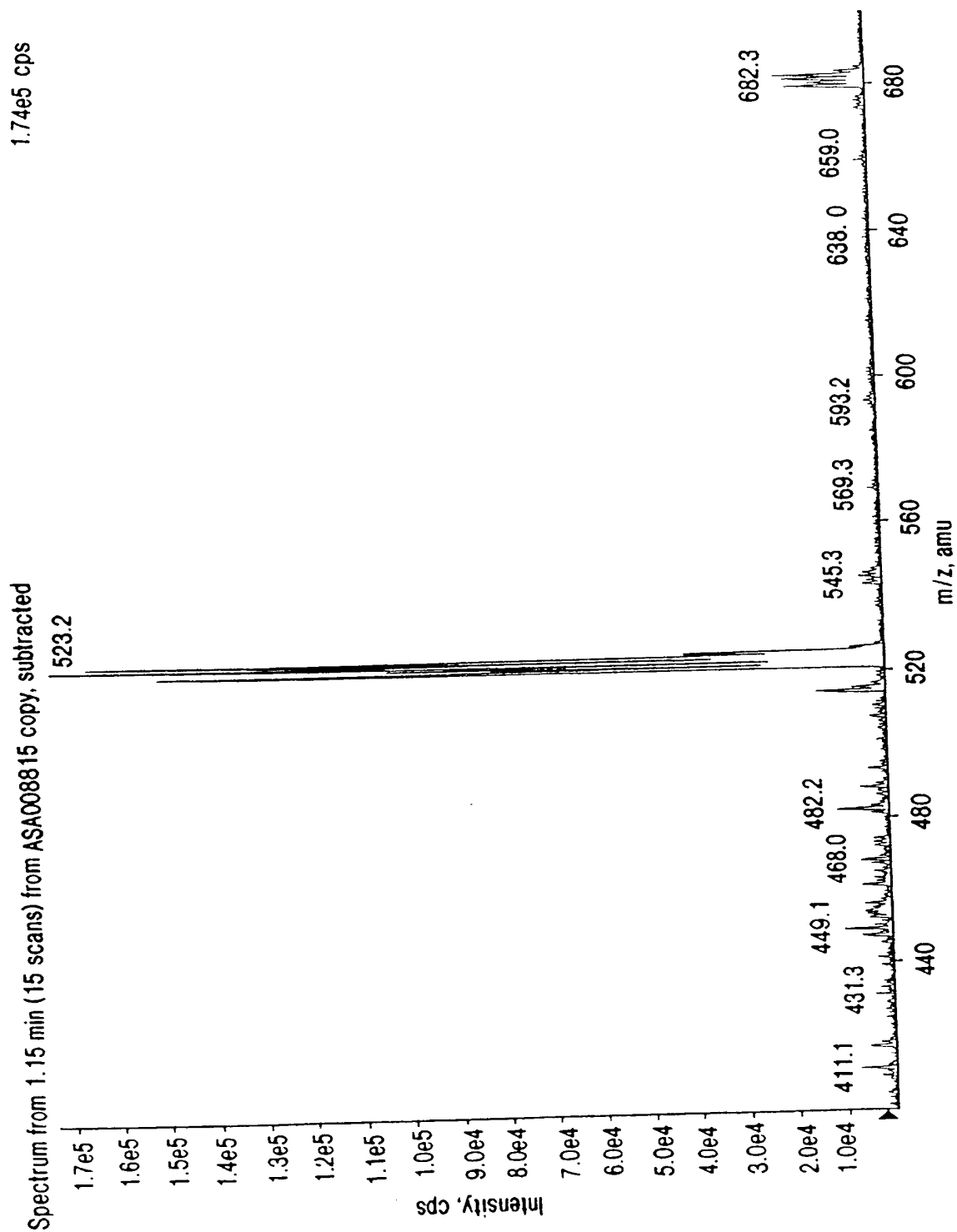


FIG. 229

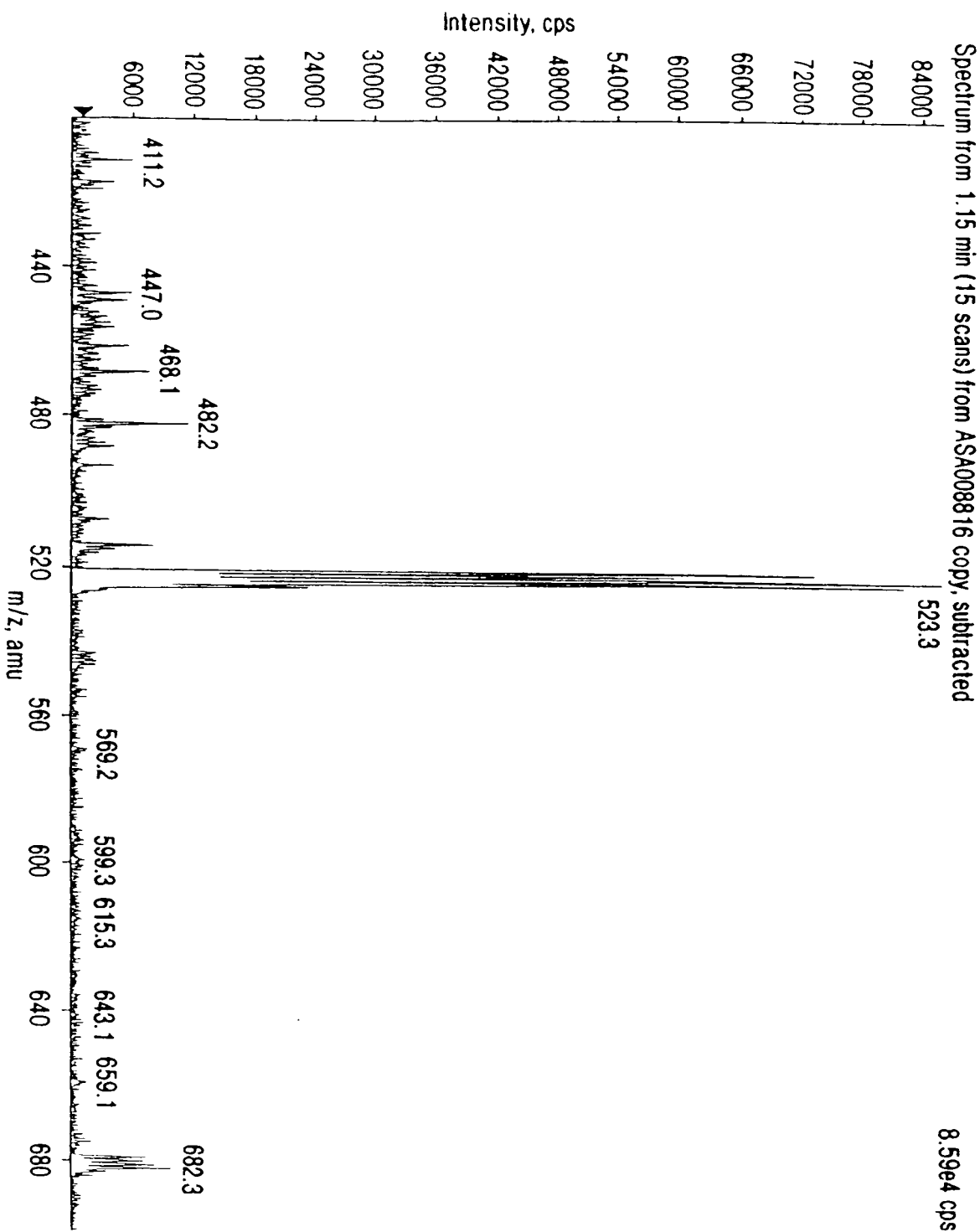


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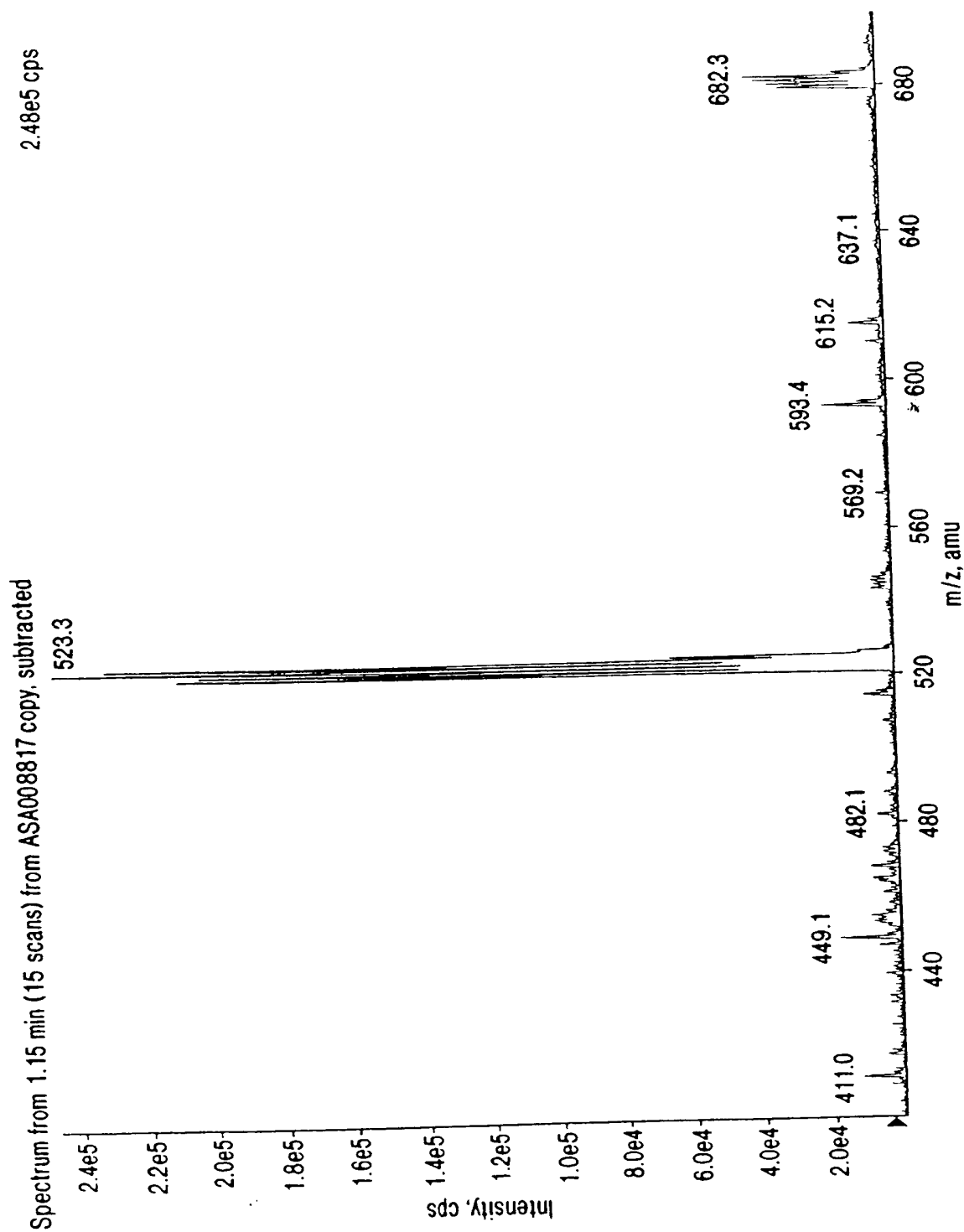


FIG. 231

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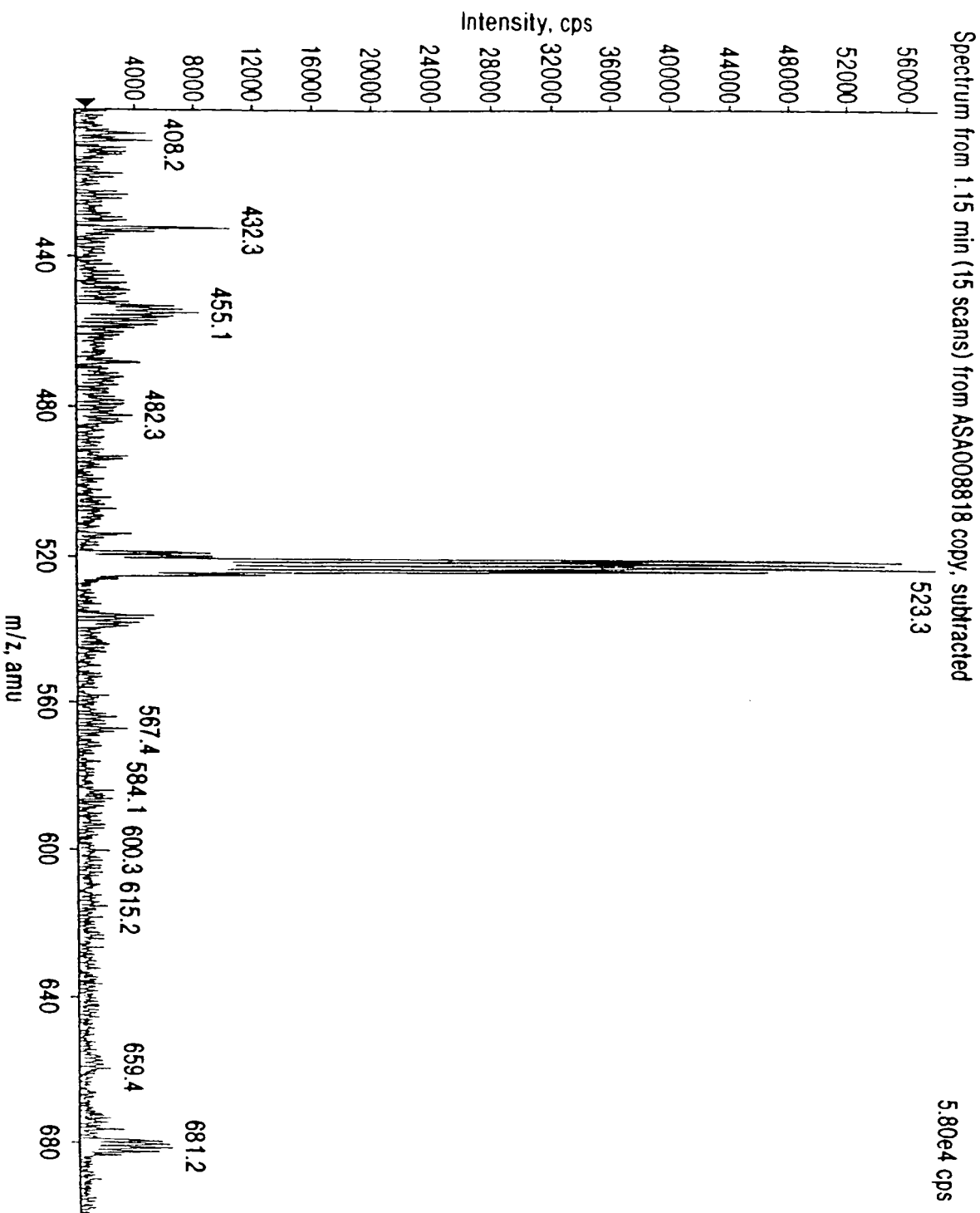


FIG. 232

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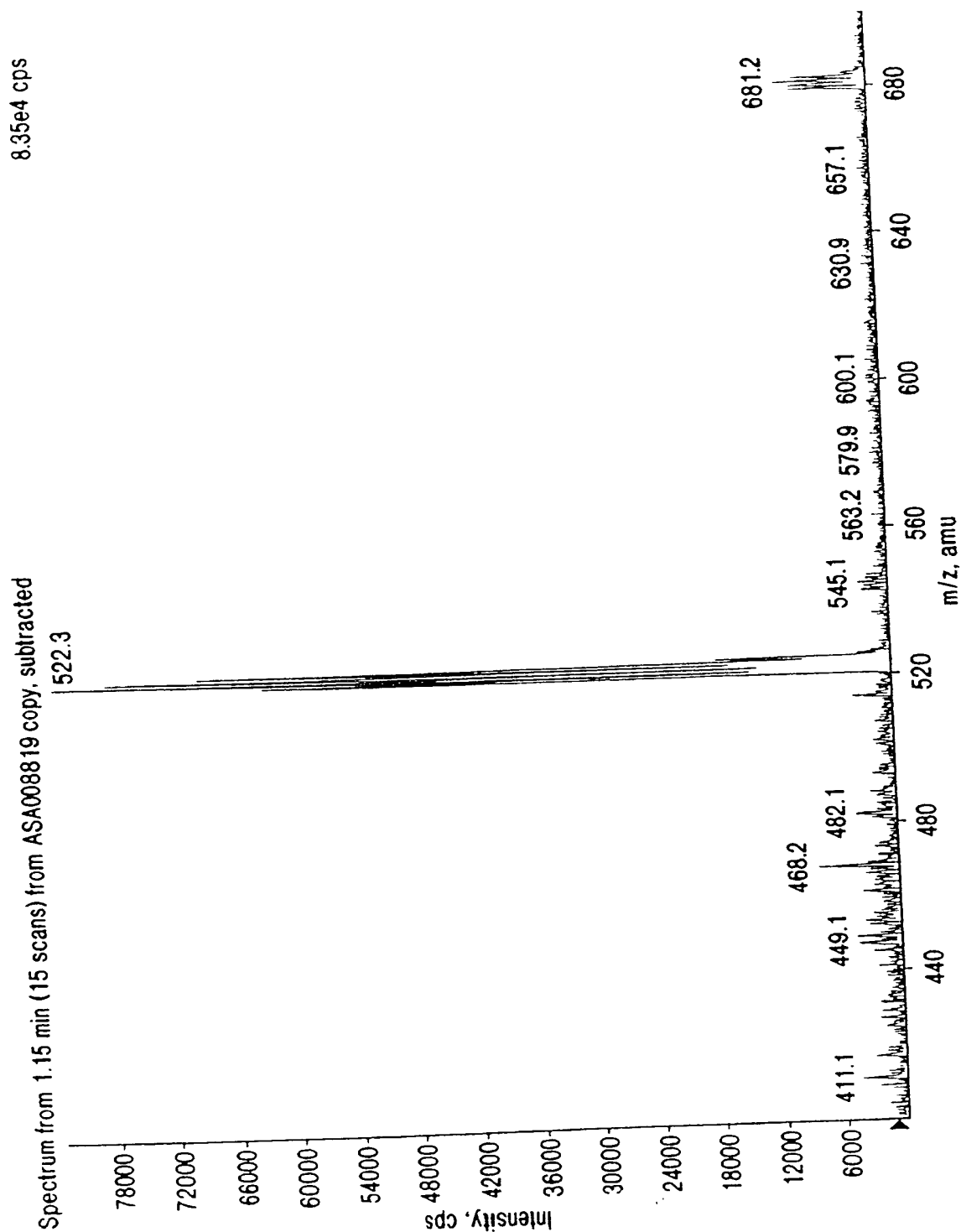


FIG. 233

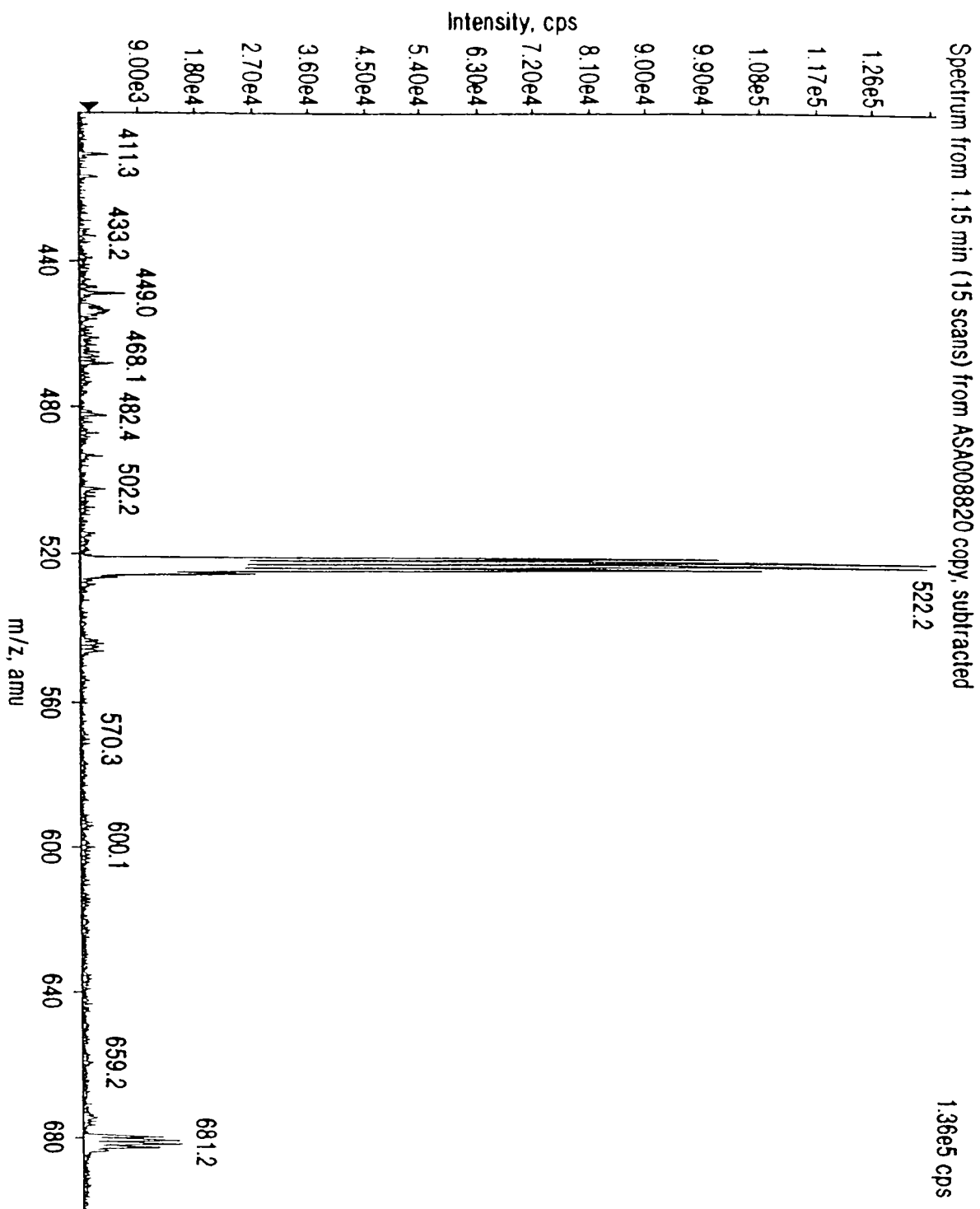


FIG. 234

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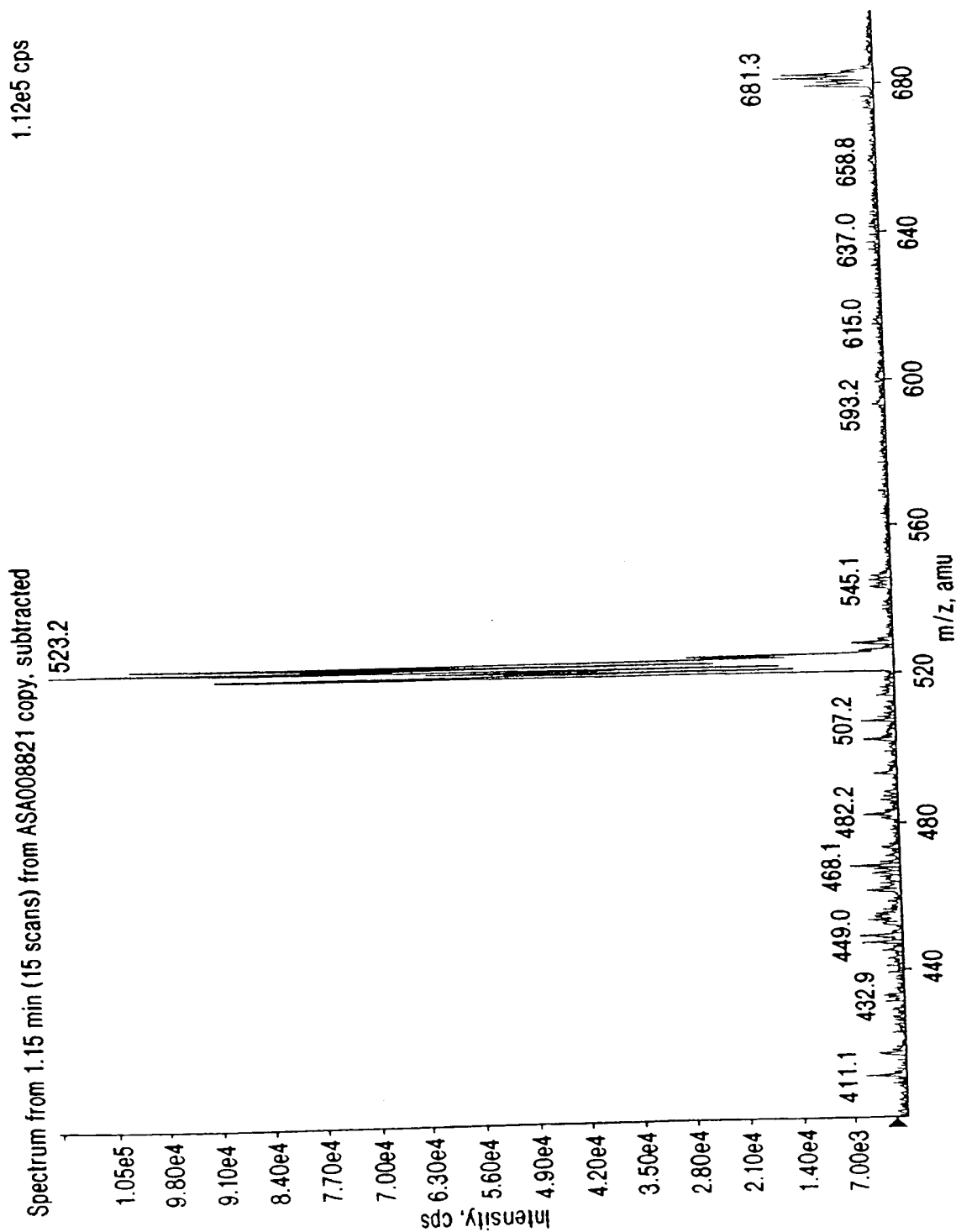


FIG. 235

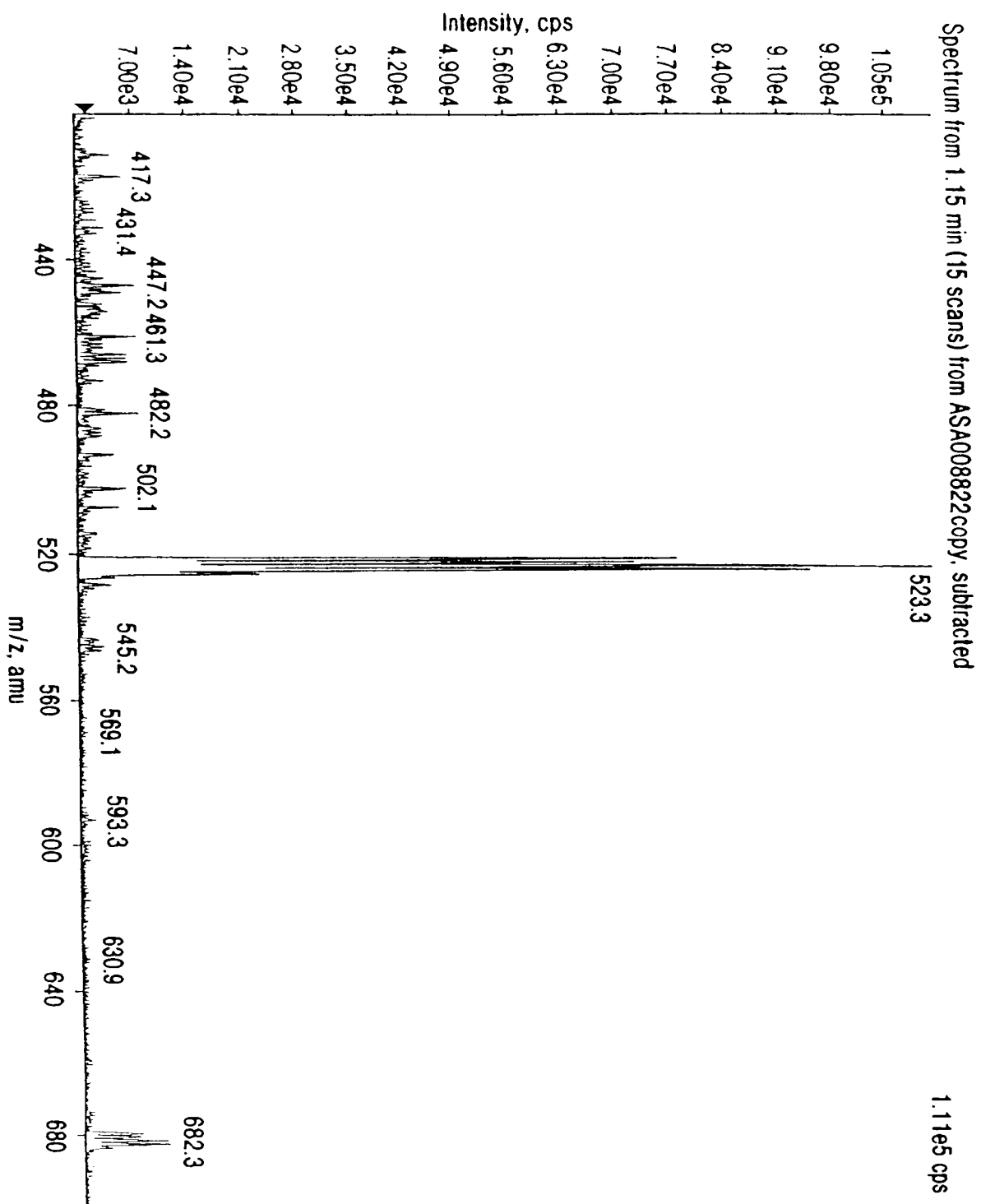


FIG. 236

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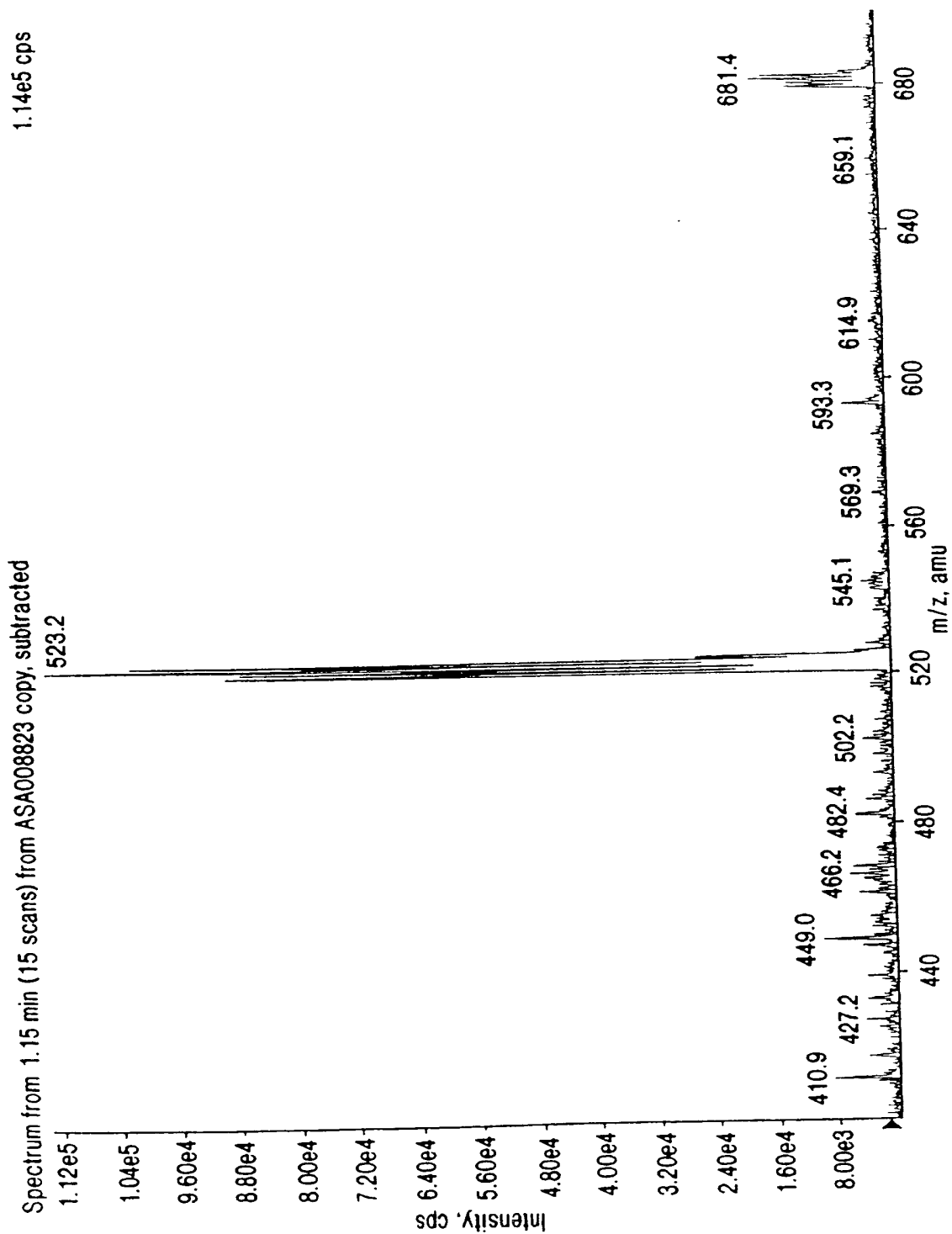


FIG. 237

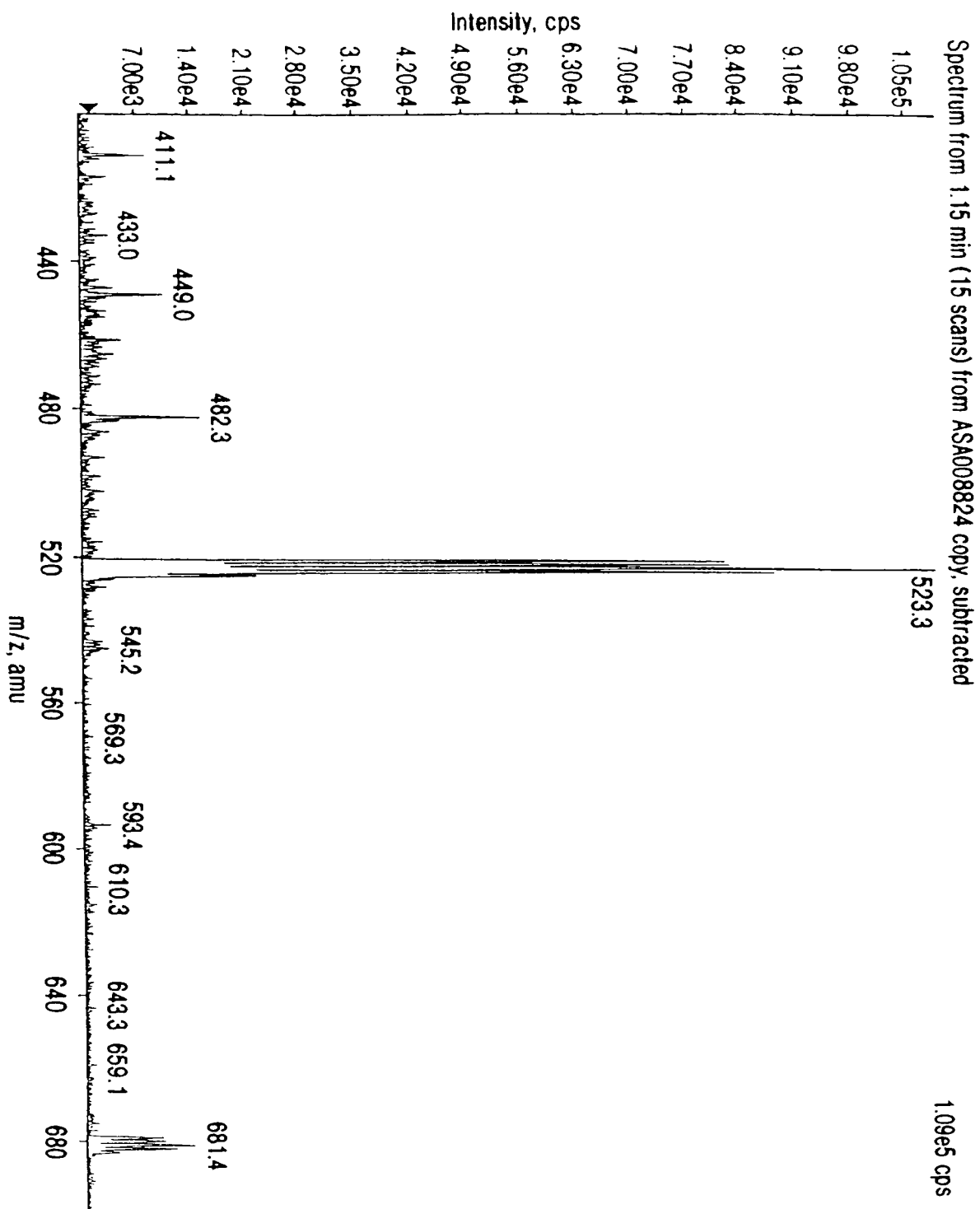


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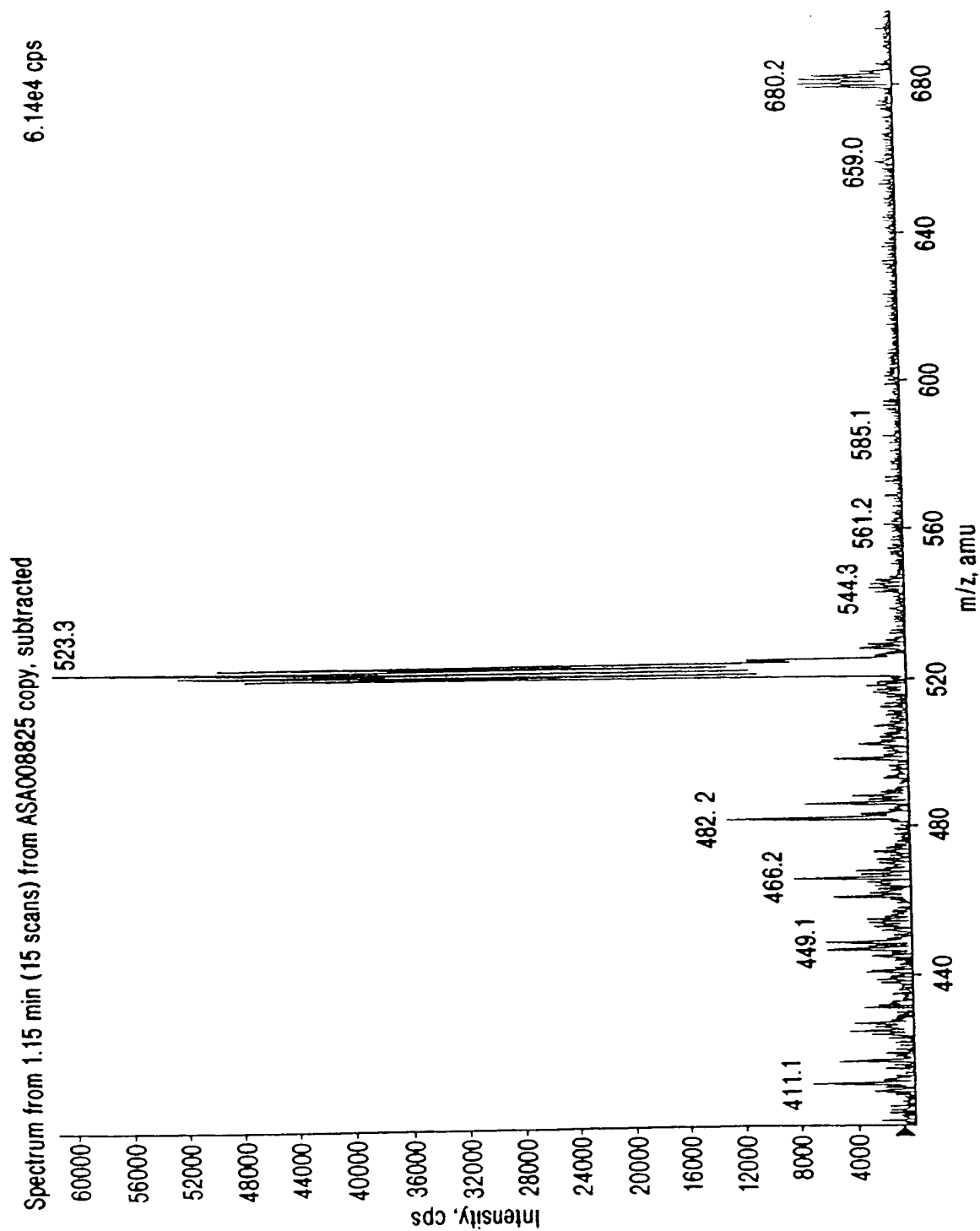


FIG. 239

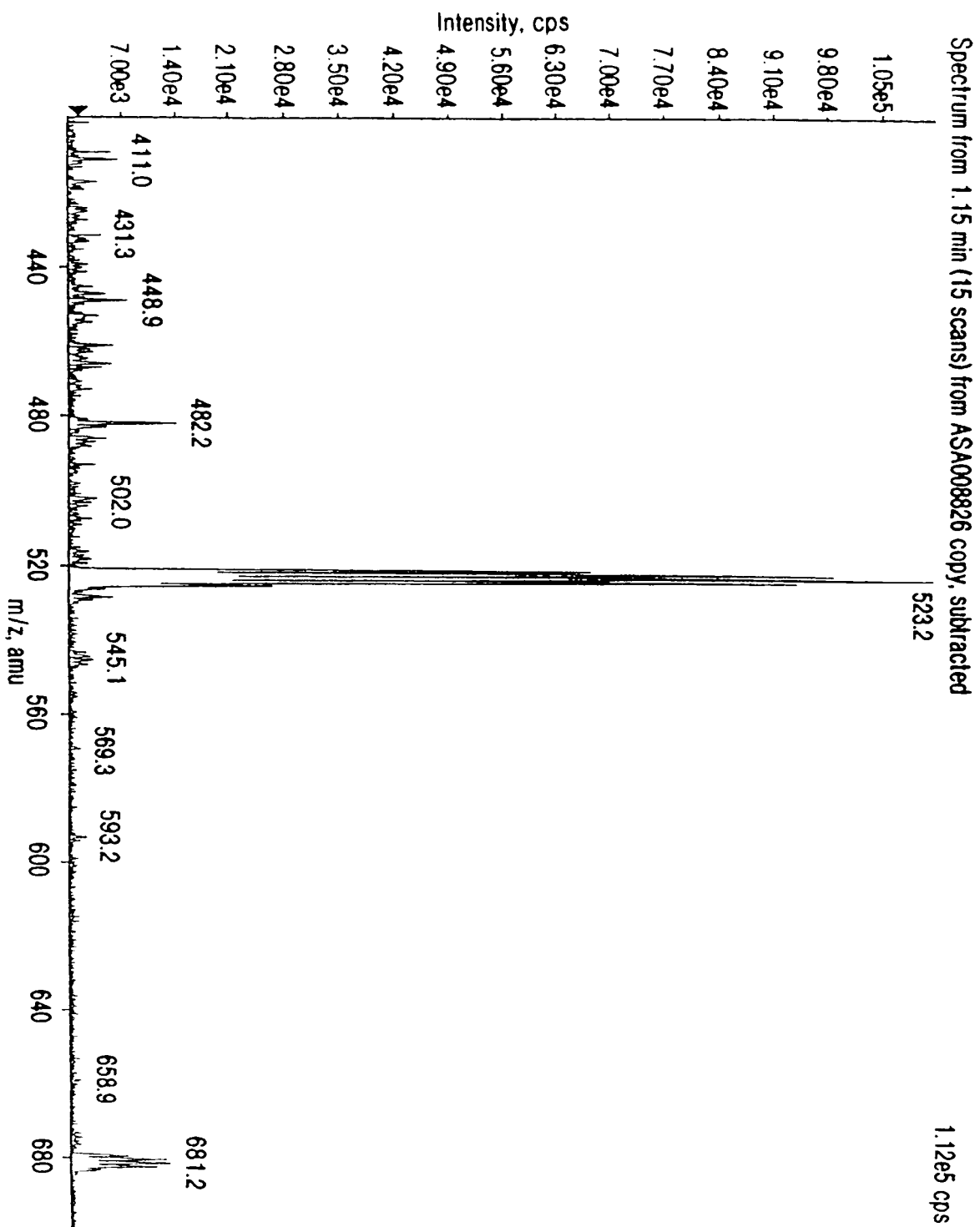


FIG. 240

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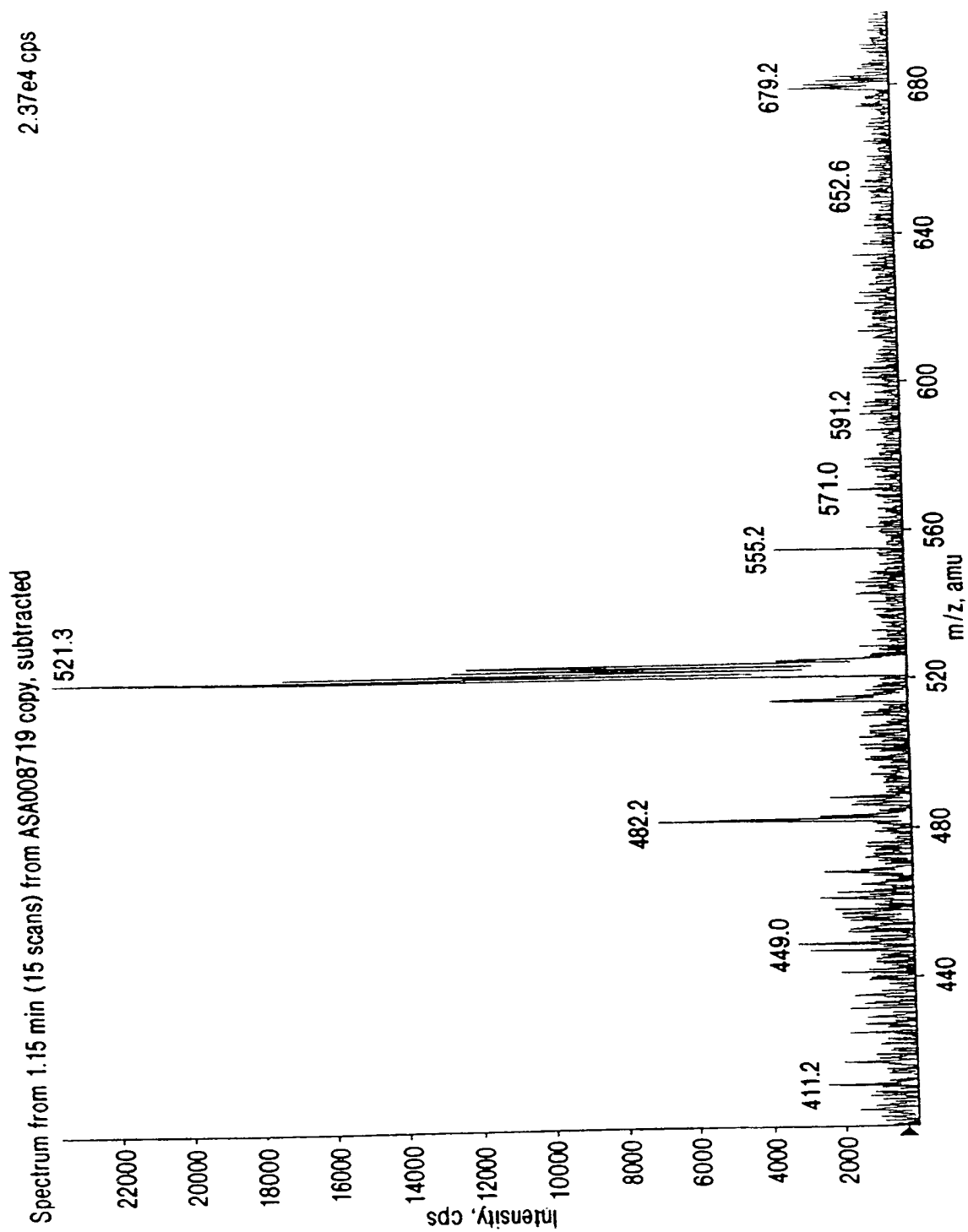


FIG. 241

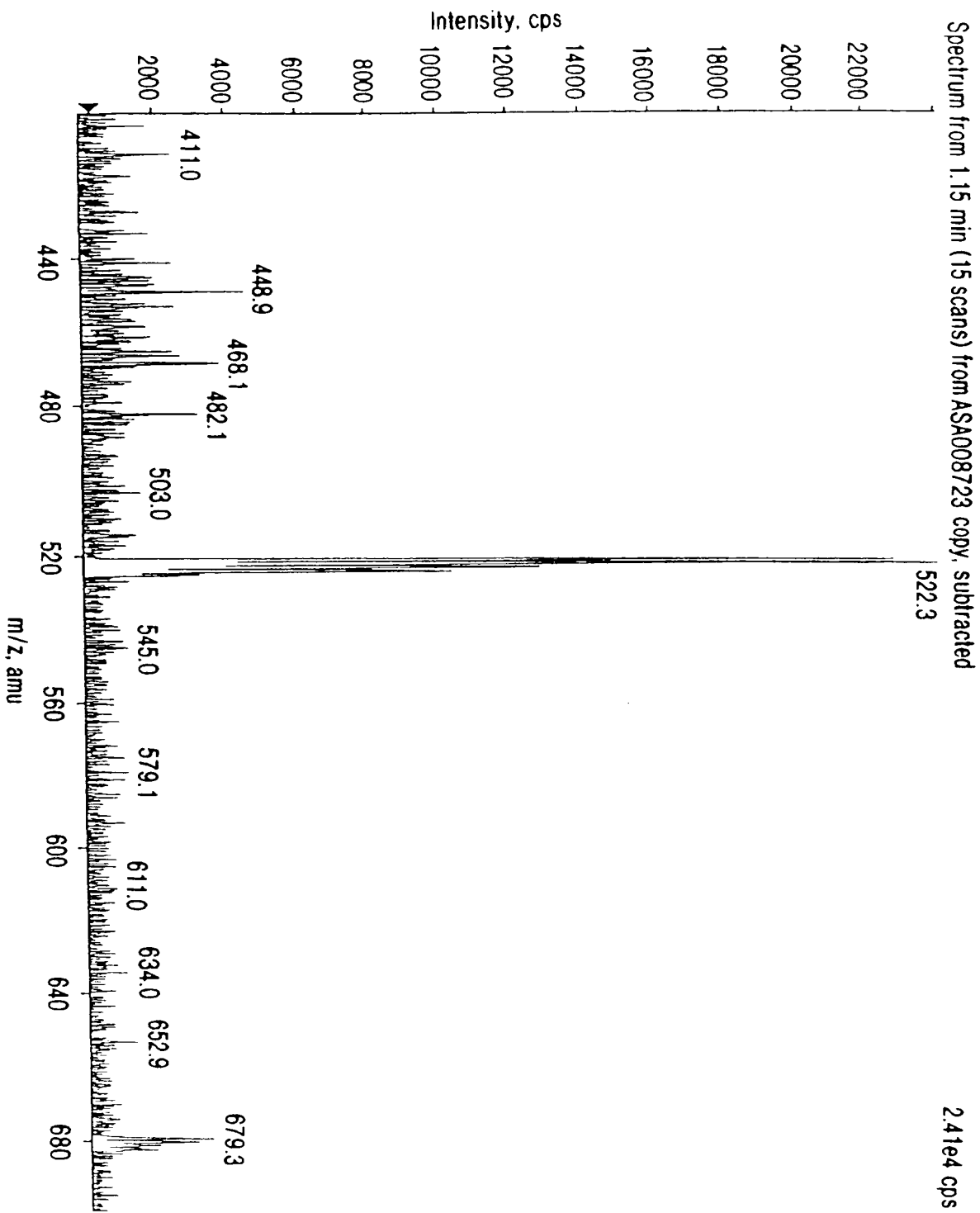


FIG. 242

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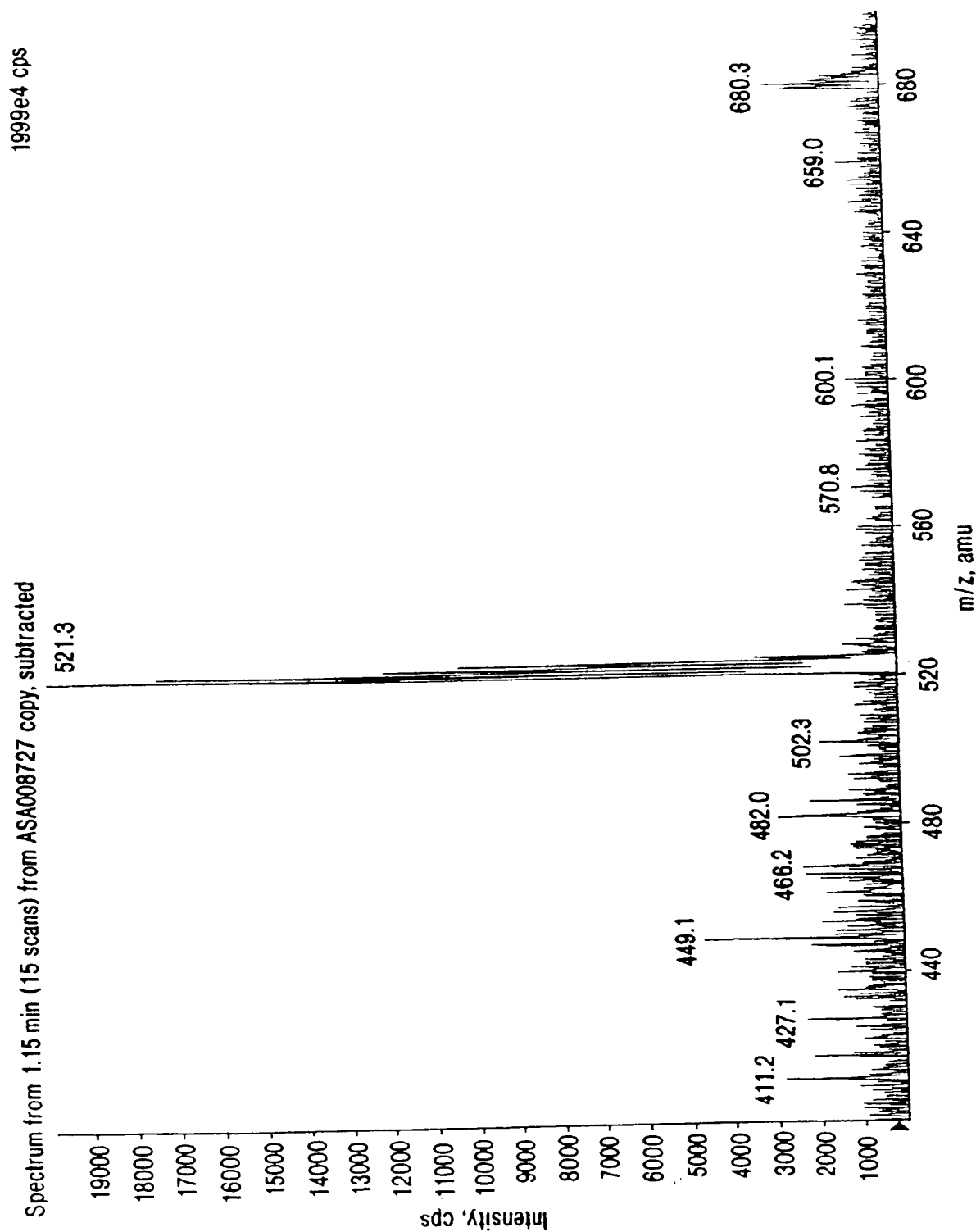


FIG. 243

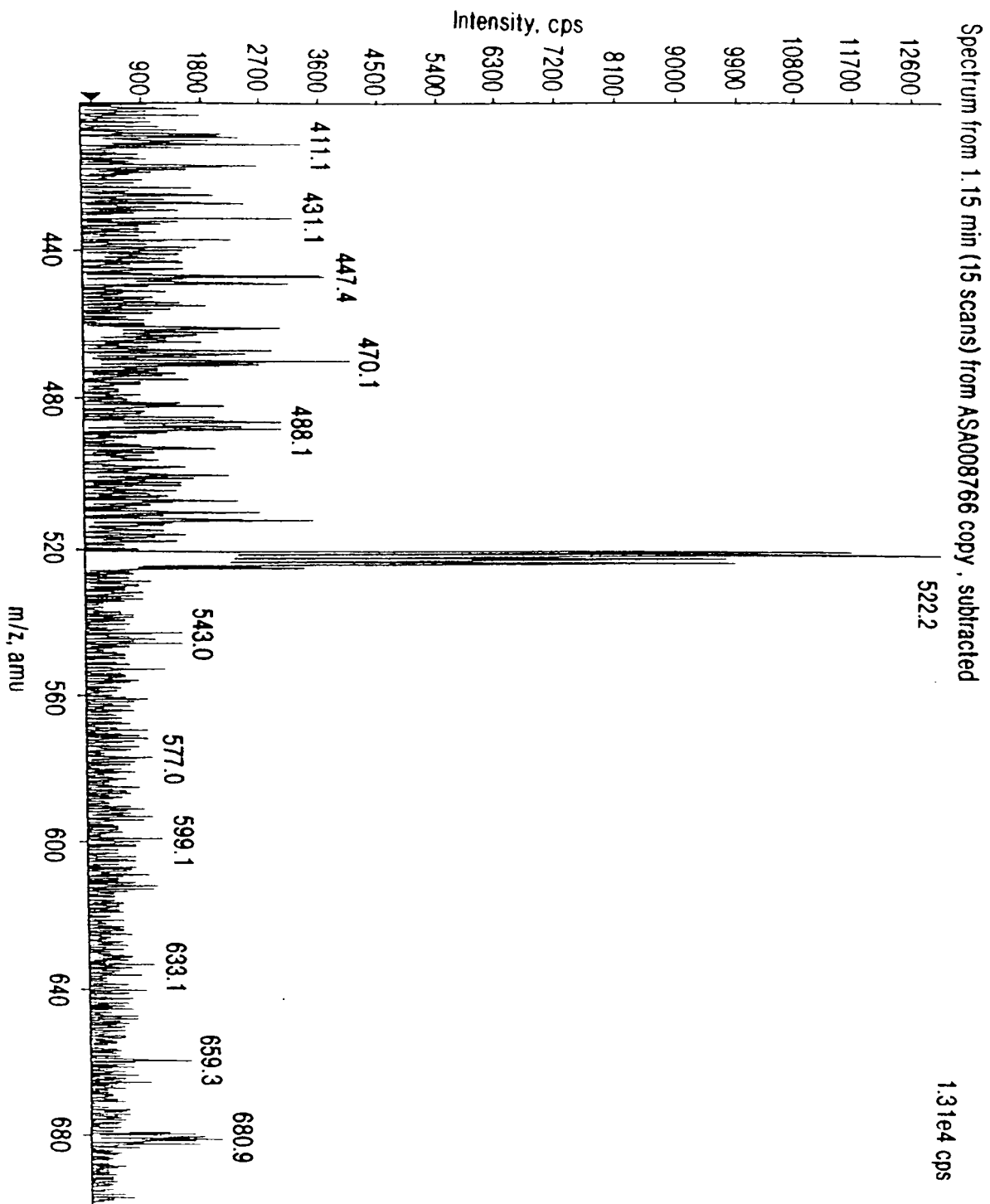


FIG. 244

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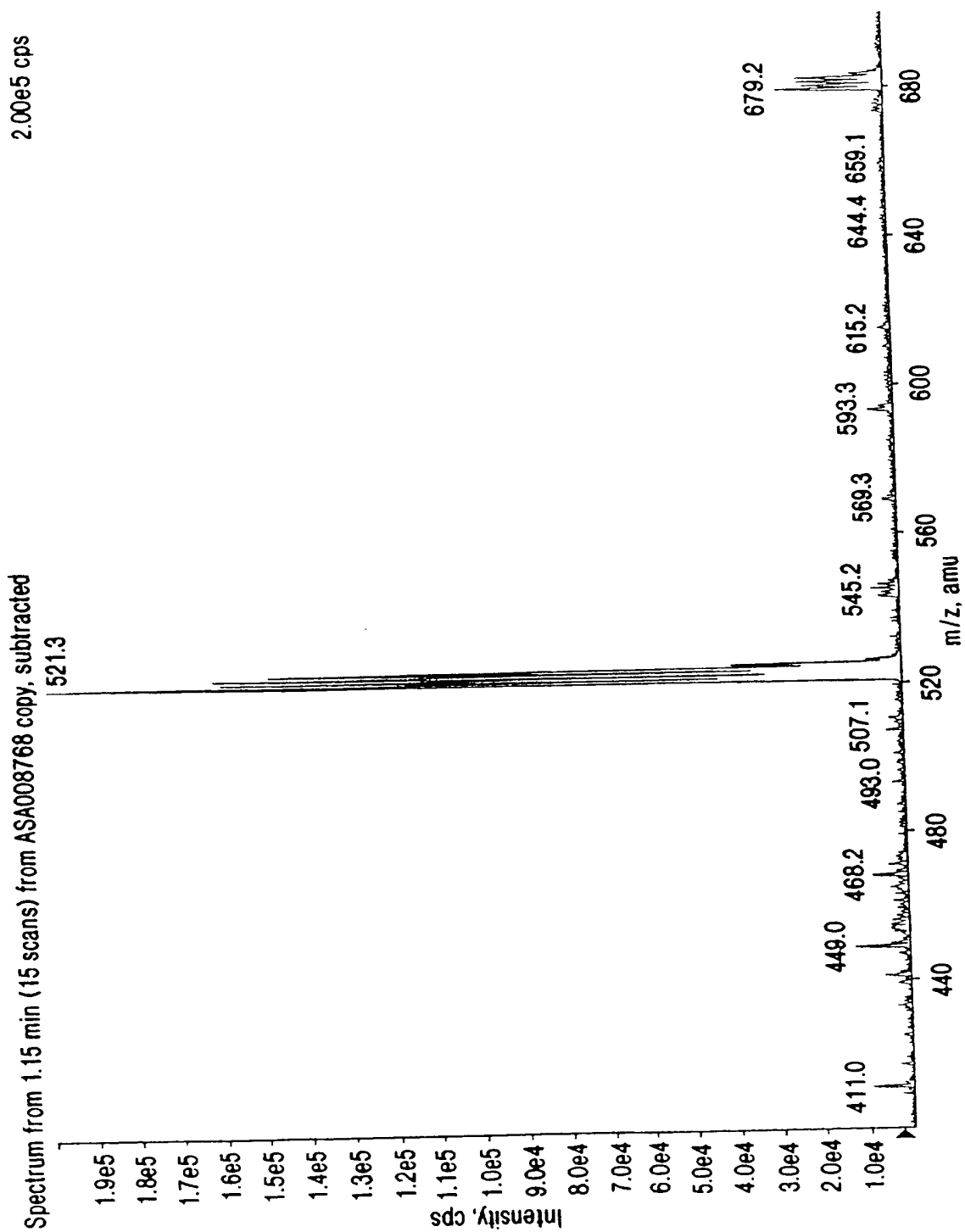


FIG. 245

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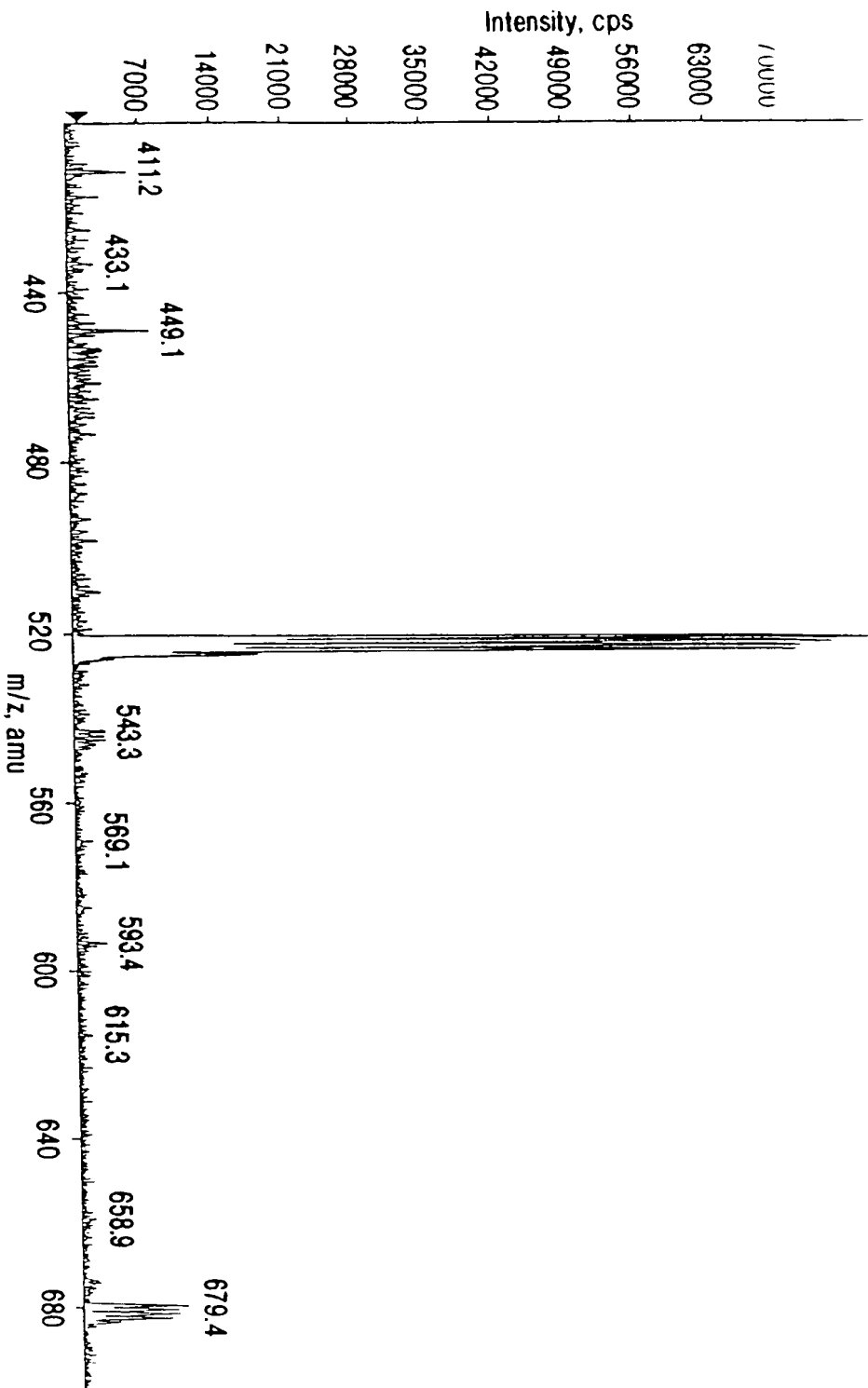


FIG. 246

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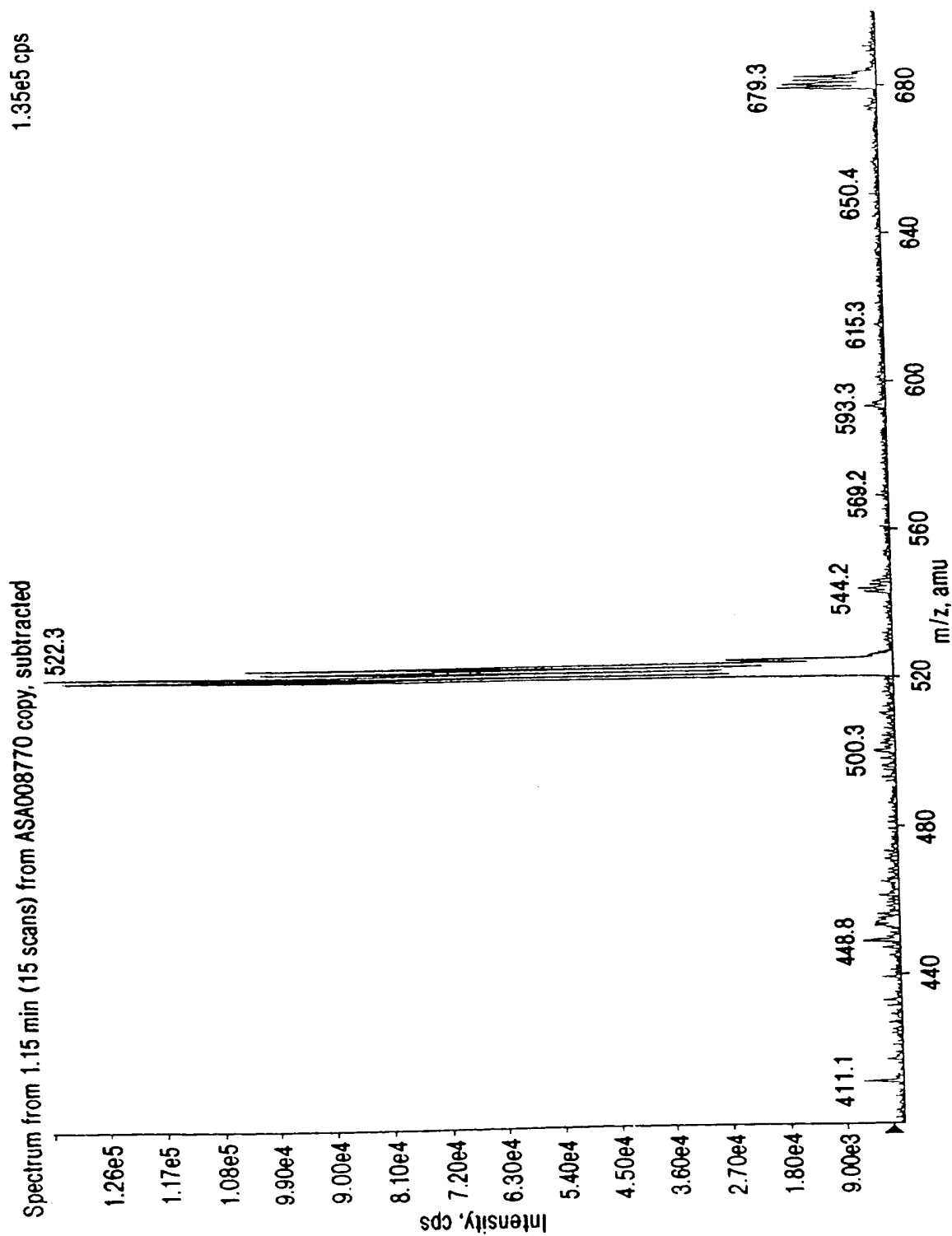


FIG. 247

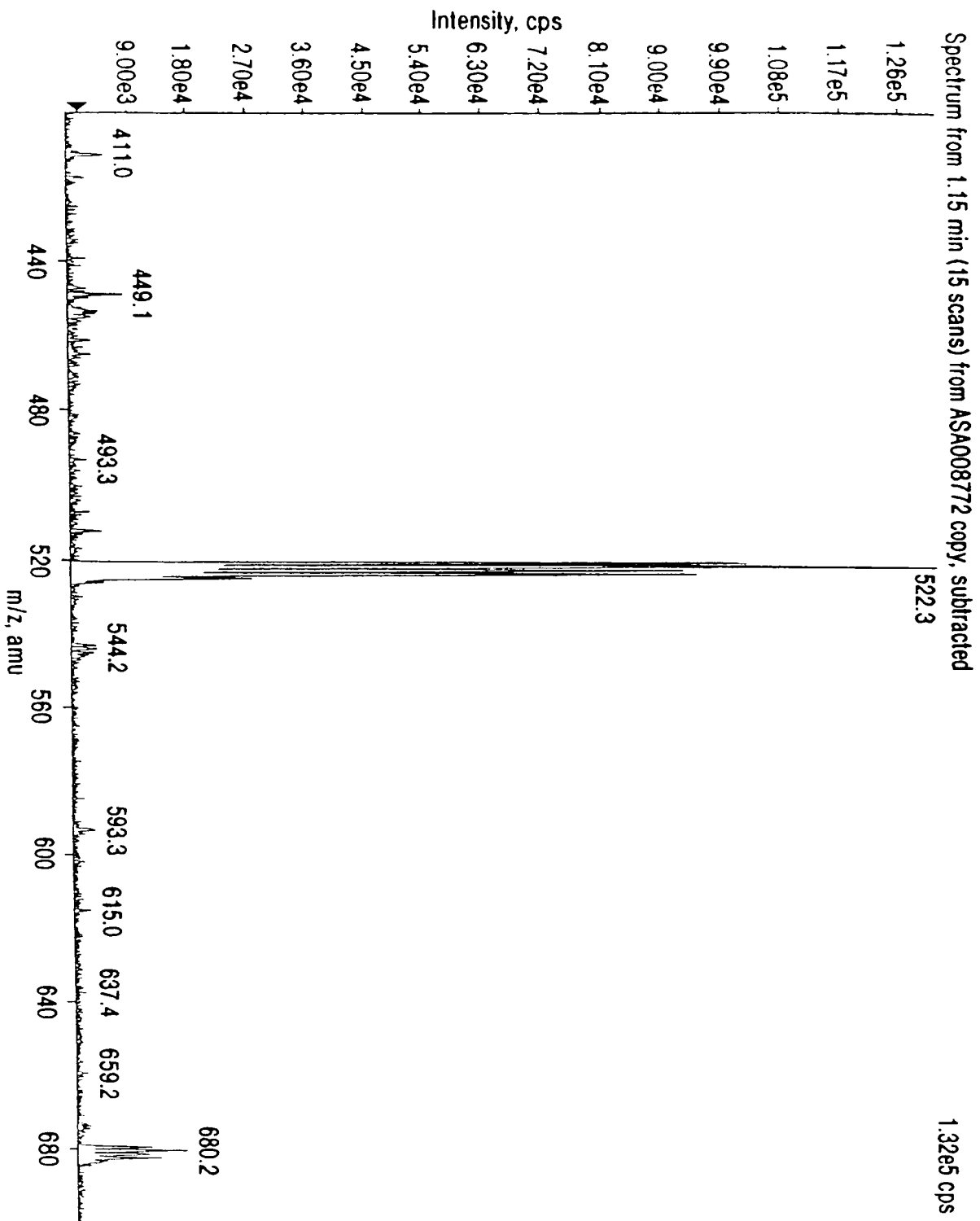


FIG. 248

1.32e5 cps

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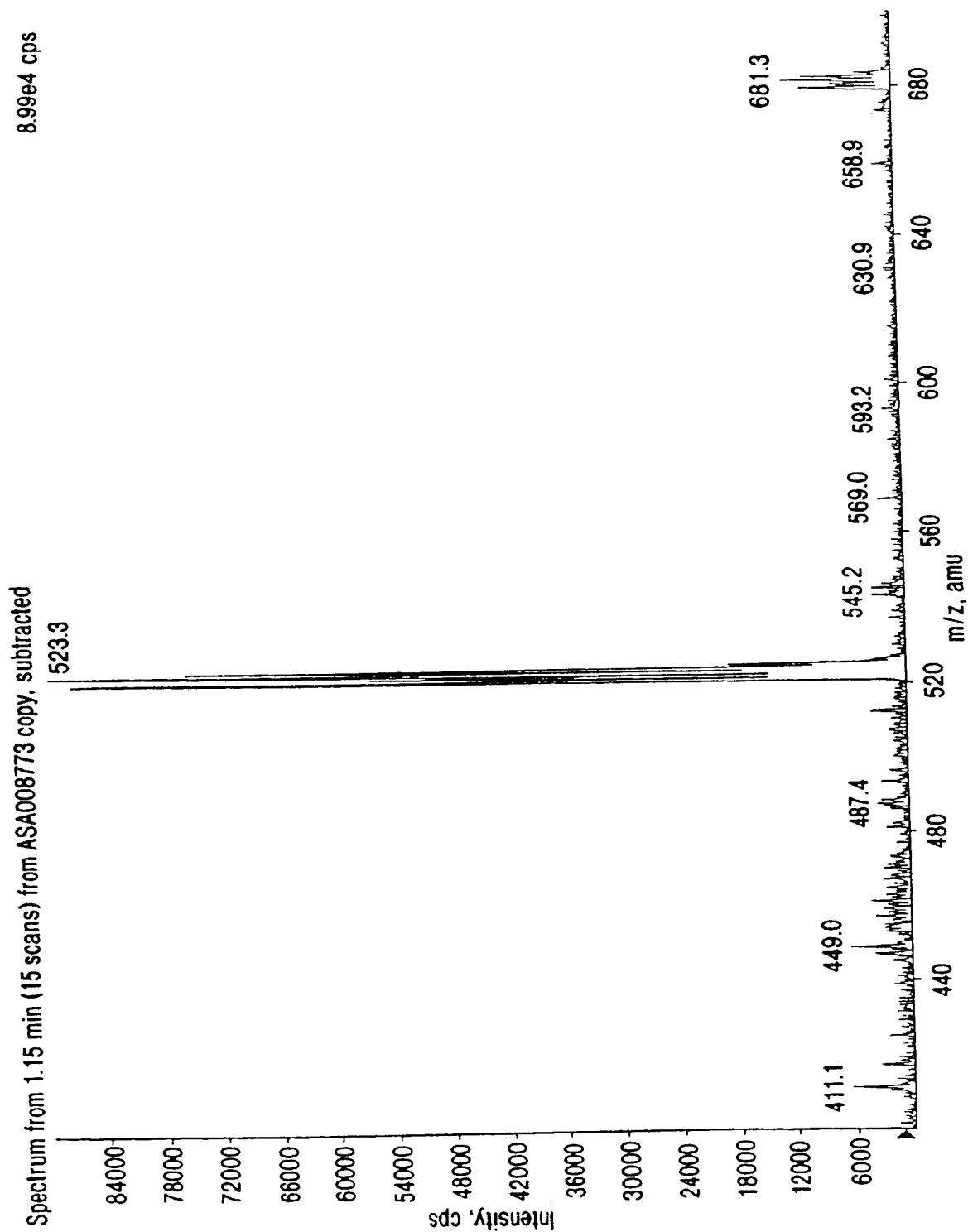


FIG. 249

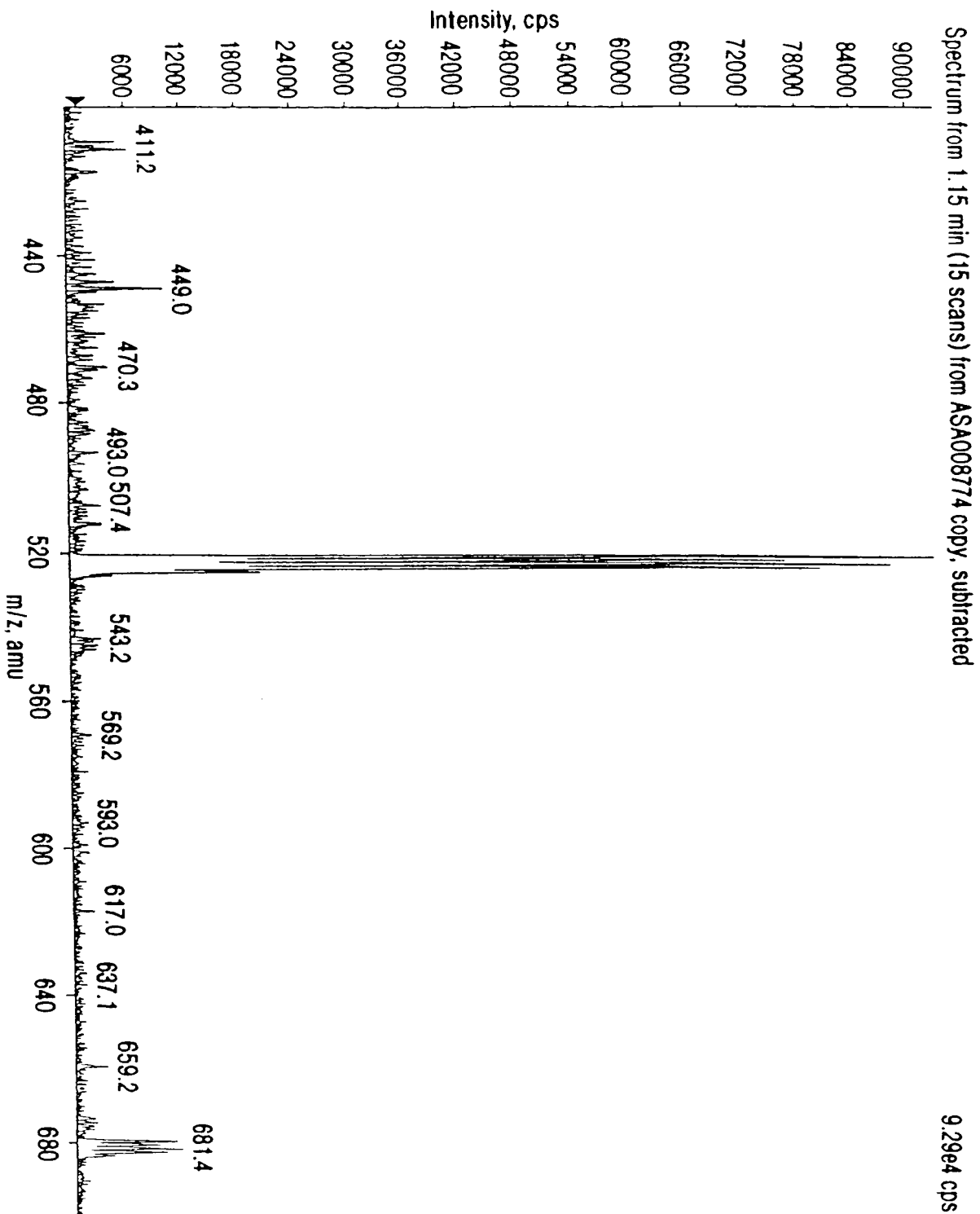


FIG. 250

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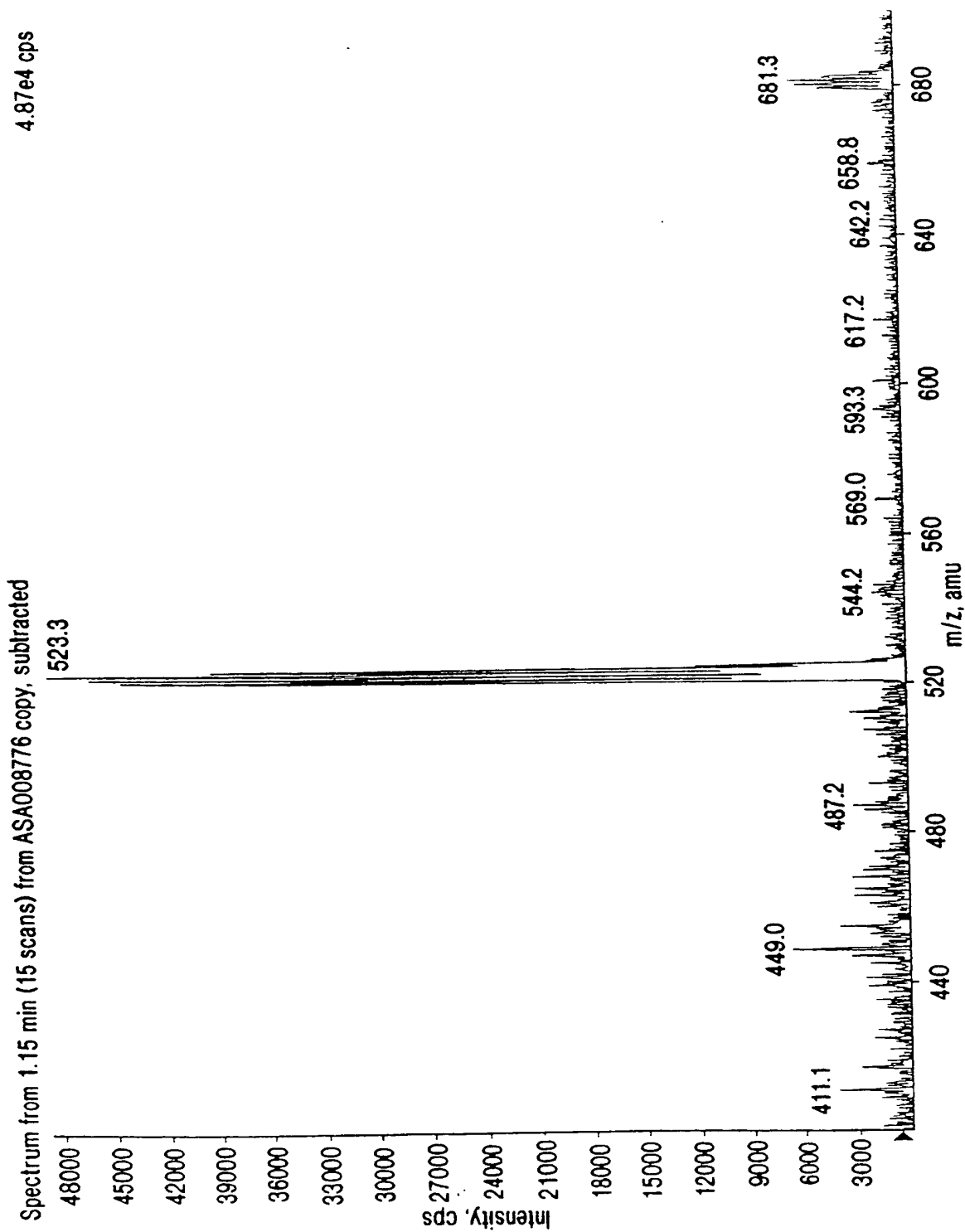


FIG. 251

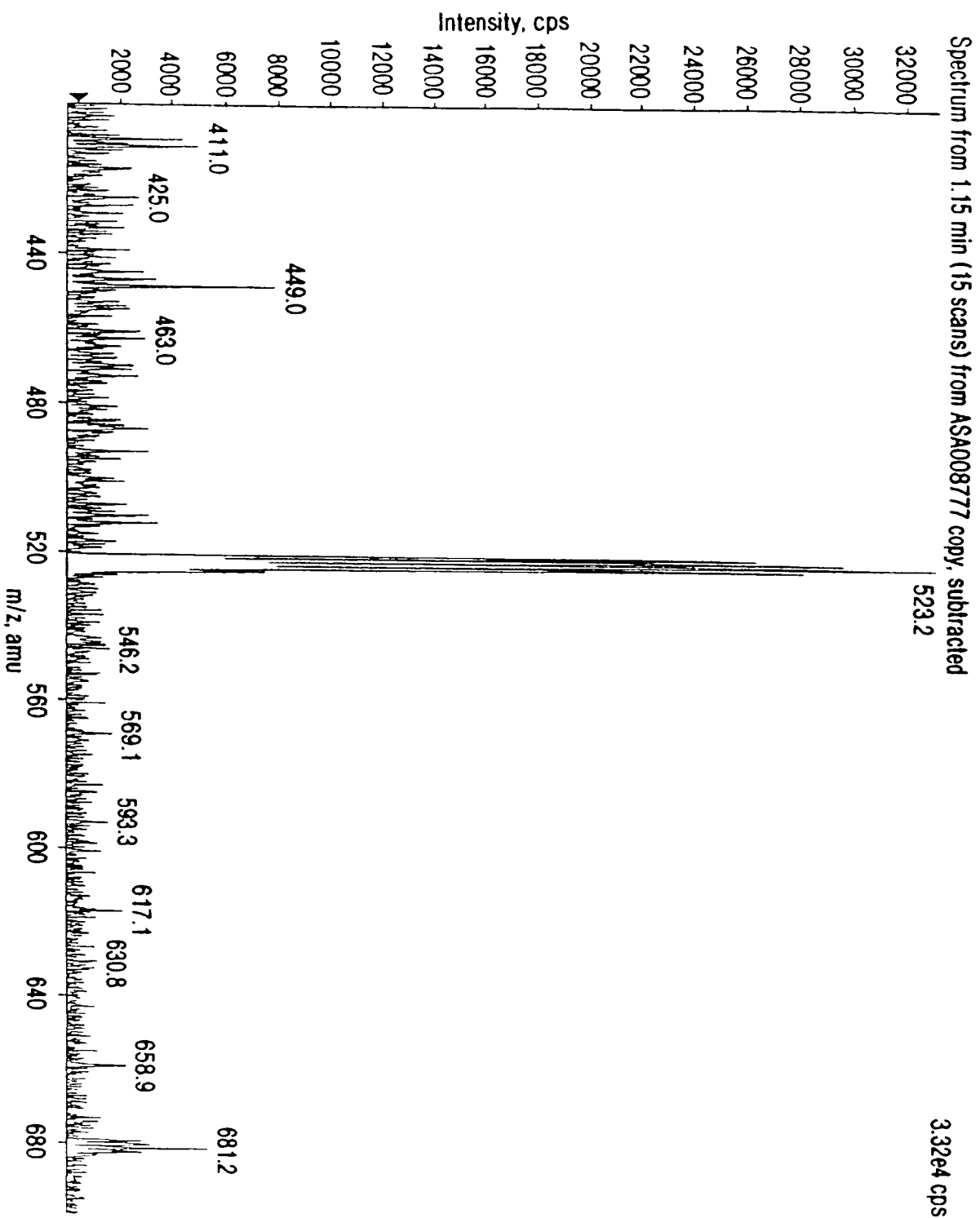


FIG. 252

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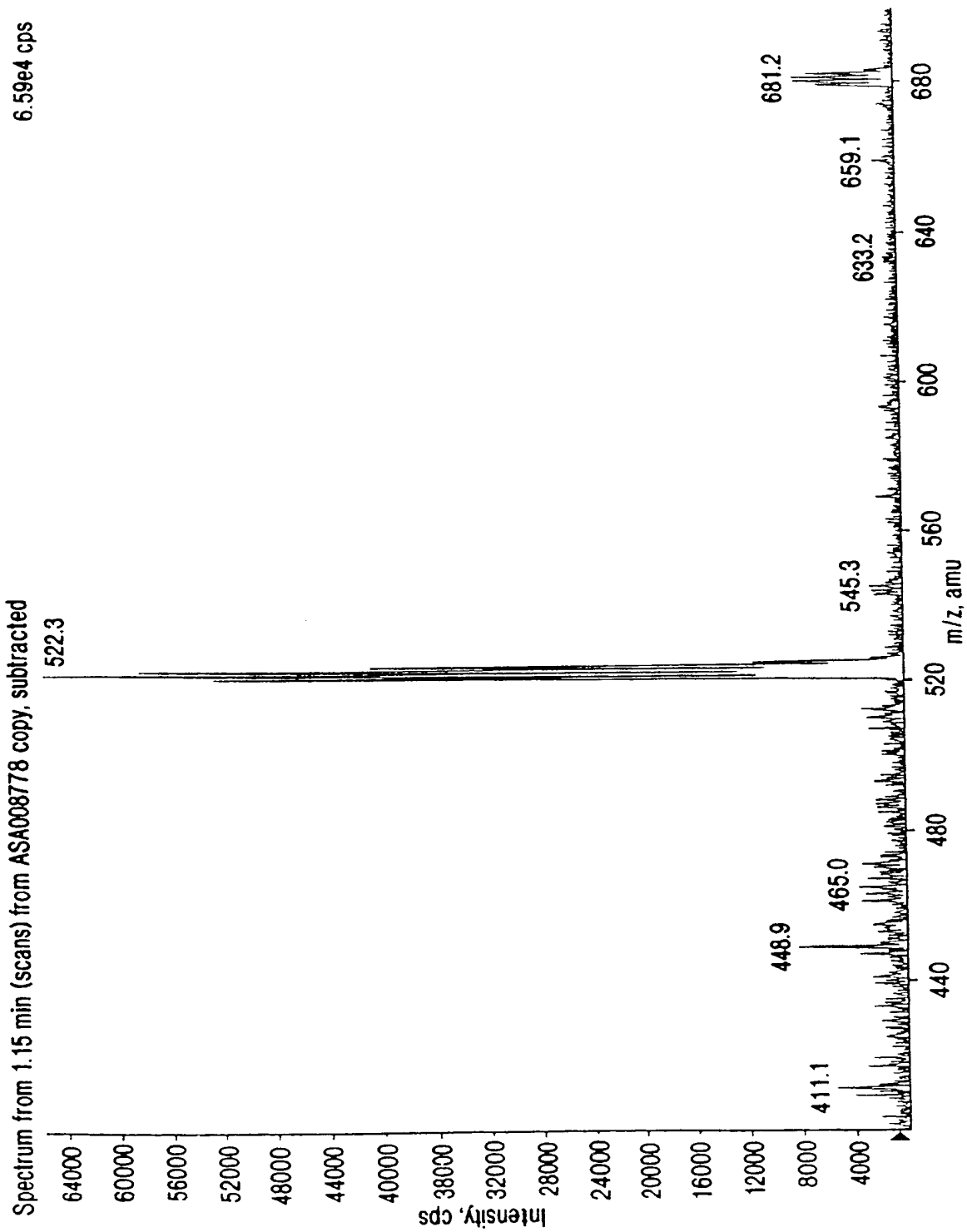


FIG. 253

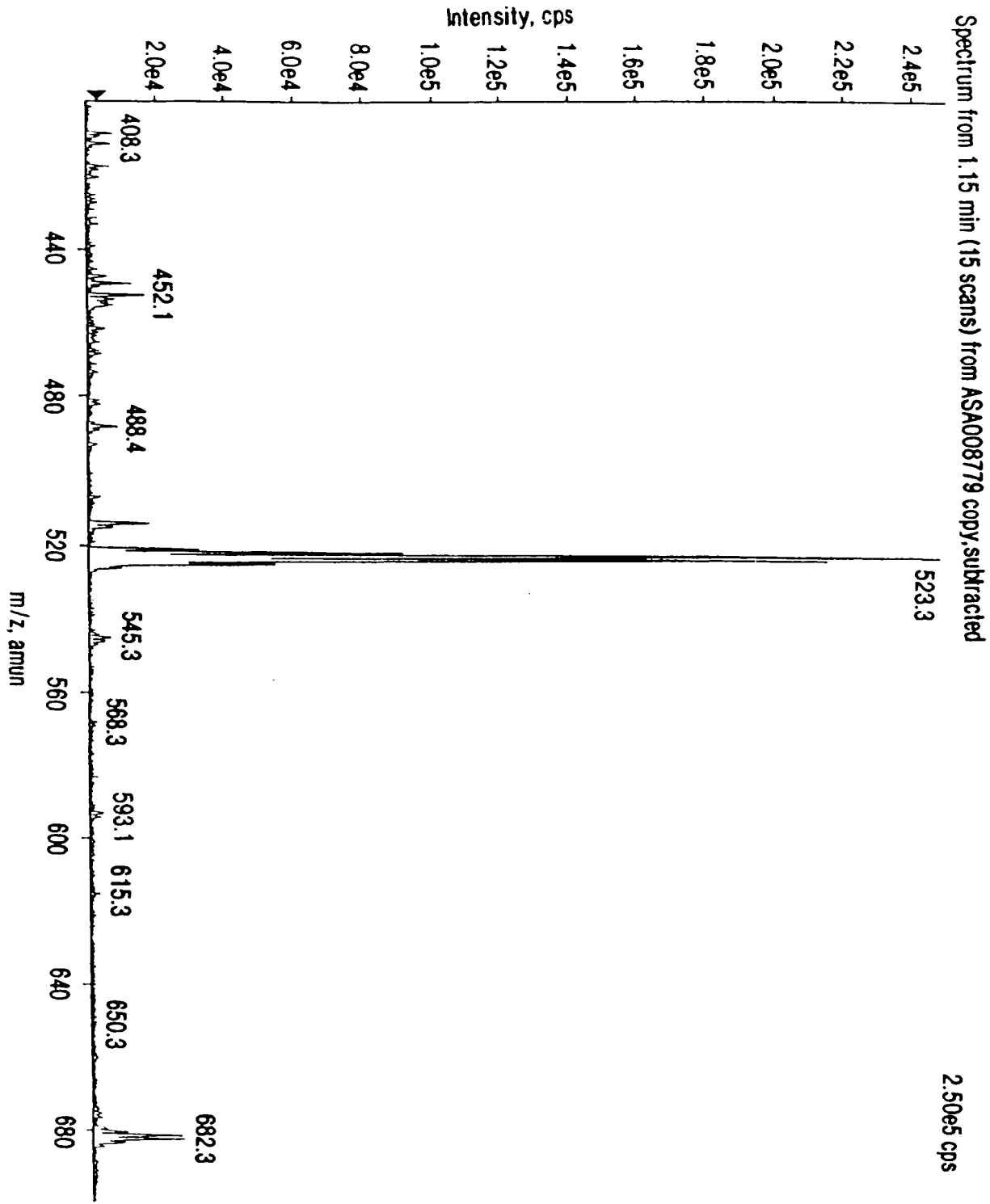


FIG. 254

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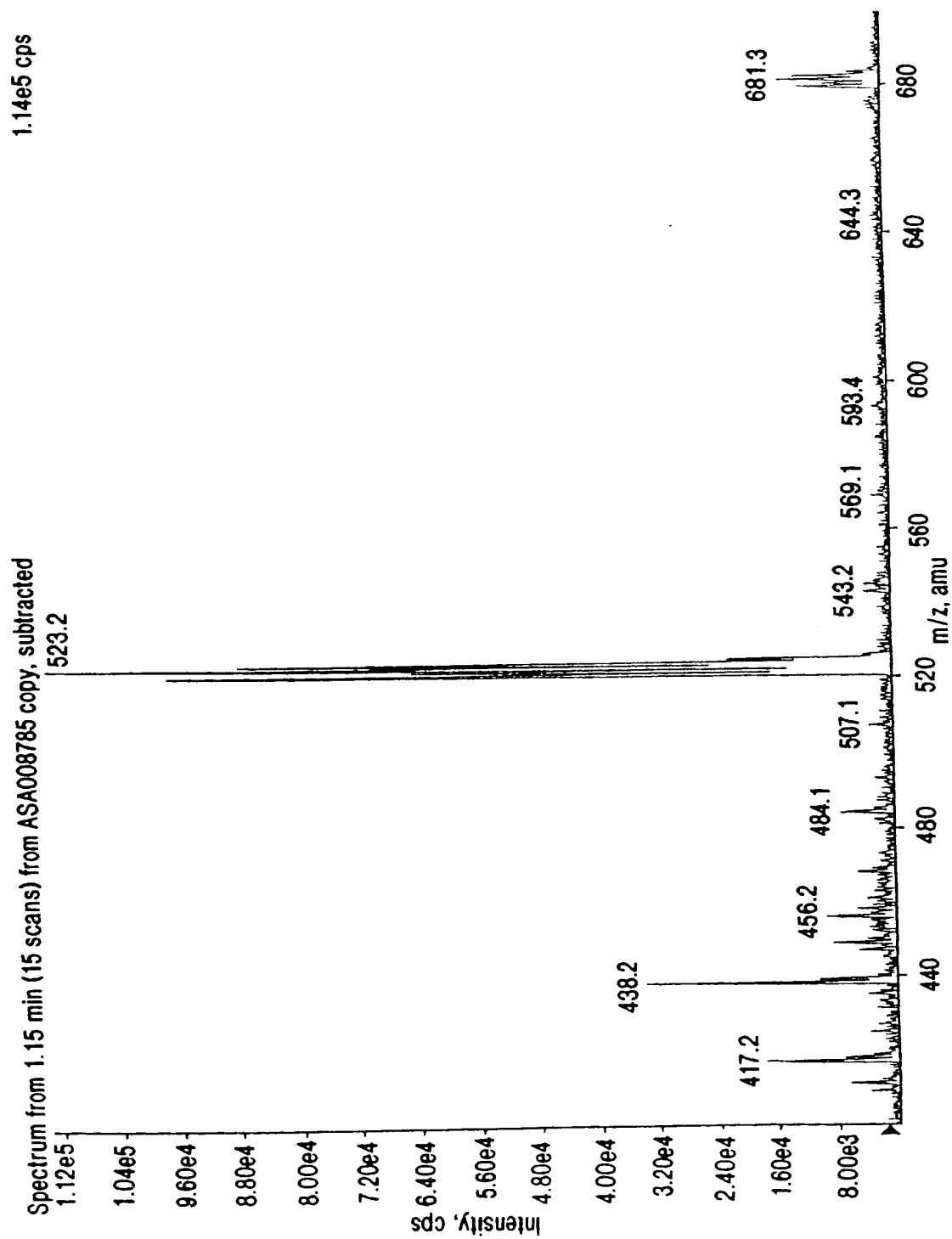


FIG. 258

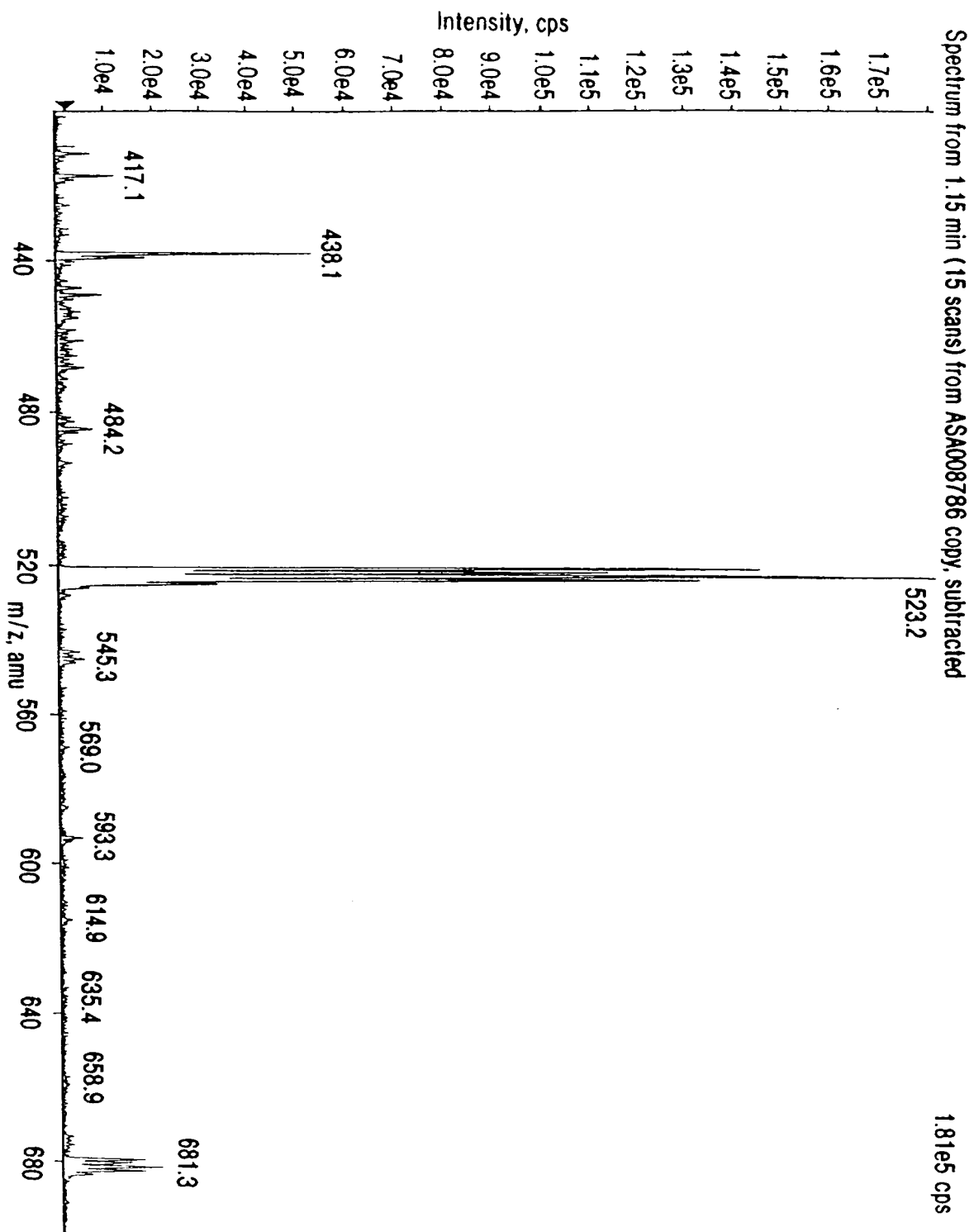


FIG. 259

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1.20e5 cps

Spectrum from 1.15 min (15 scans) from ASA008787 copy, subtracted

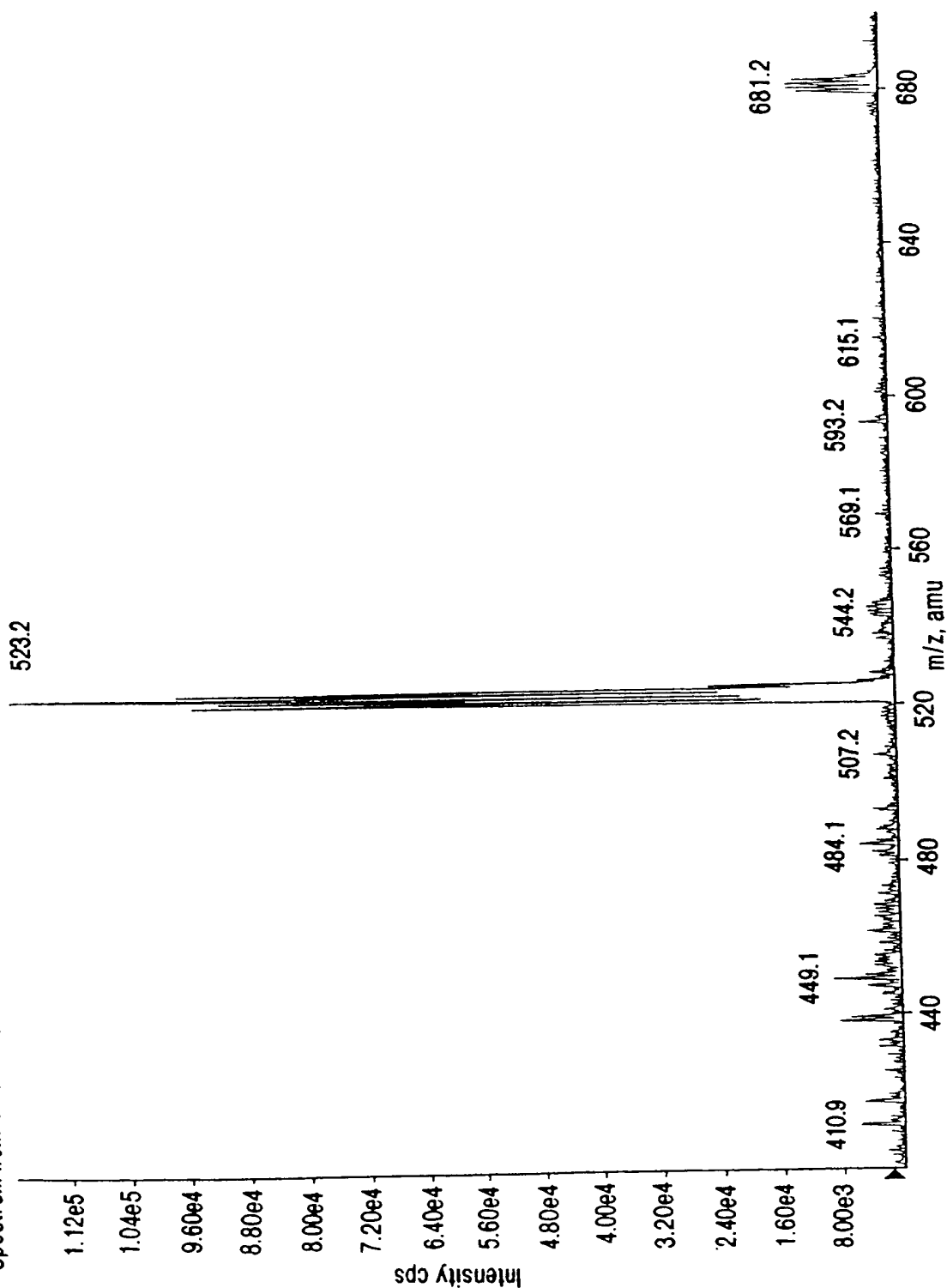


FIG. 260

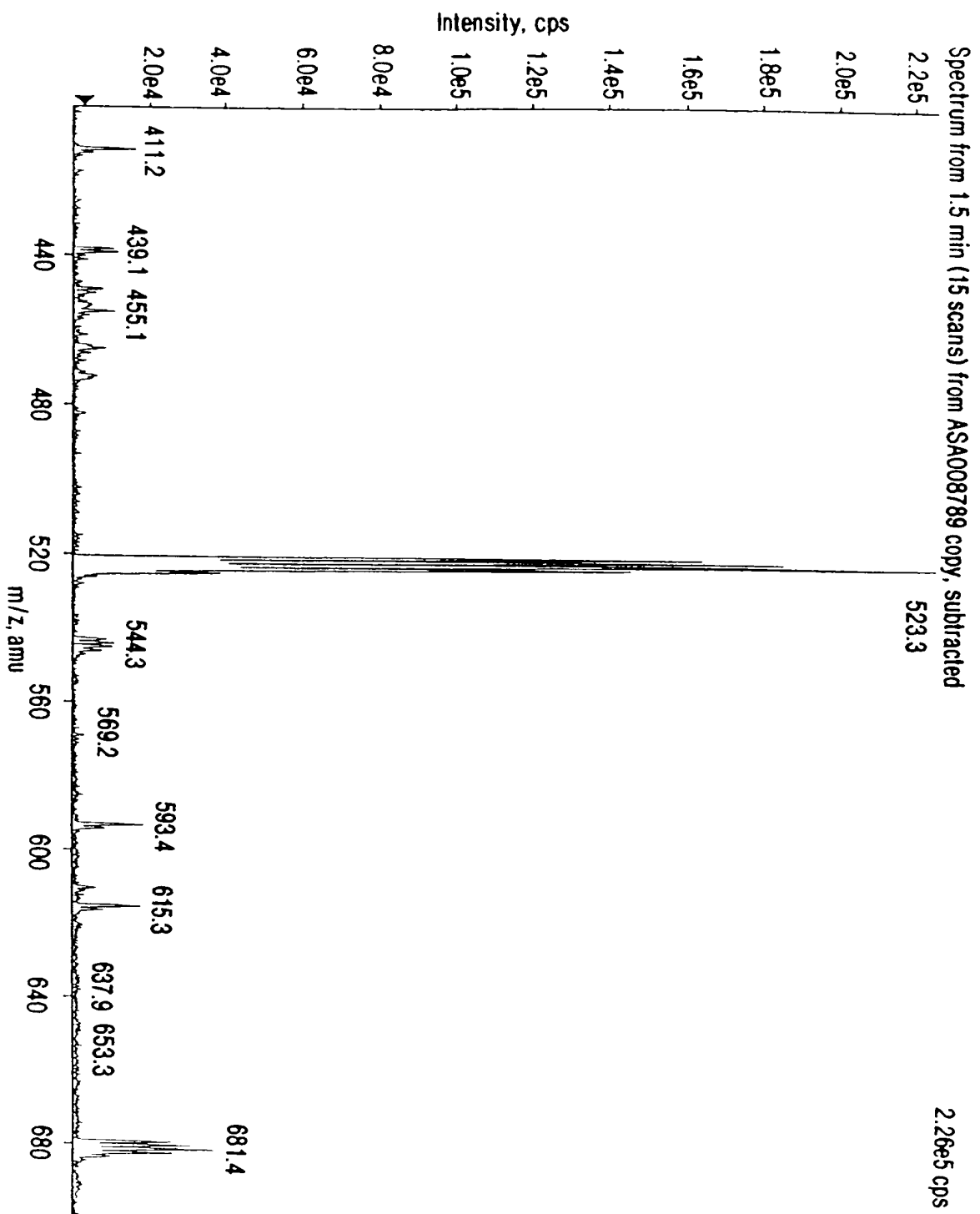


FIG. 261

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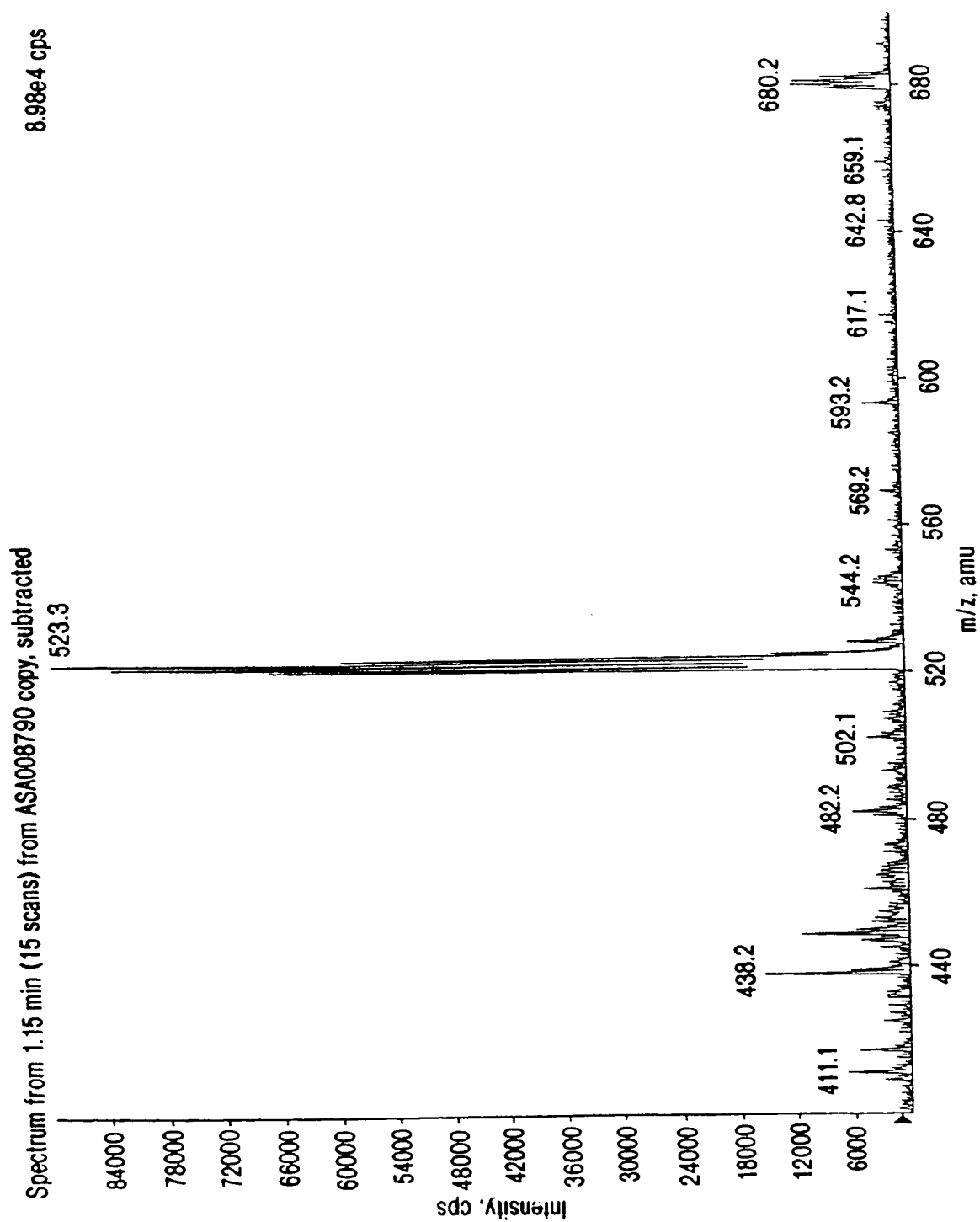


FIG. 262

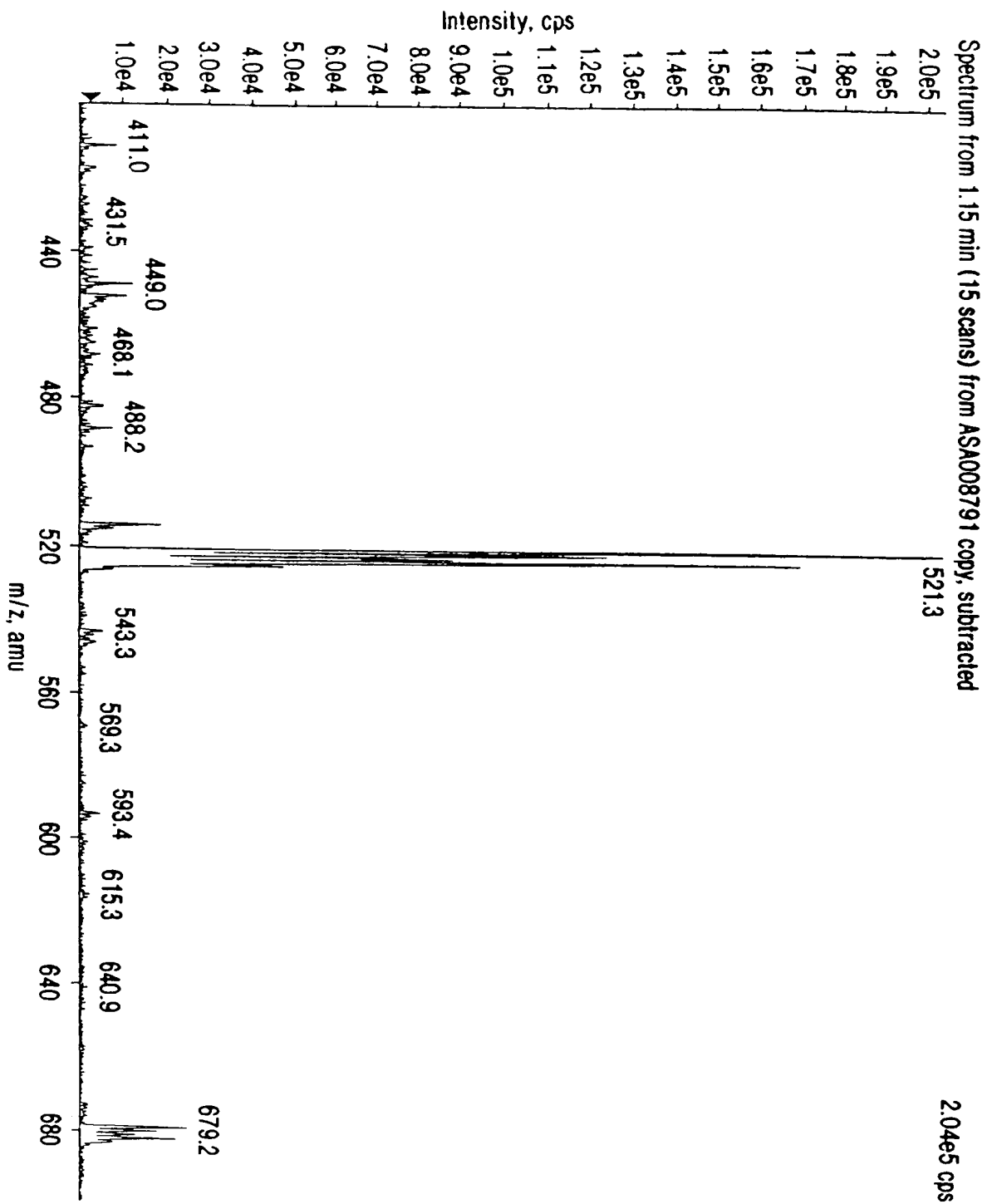


FIG. 263

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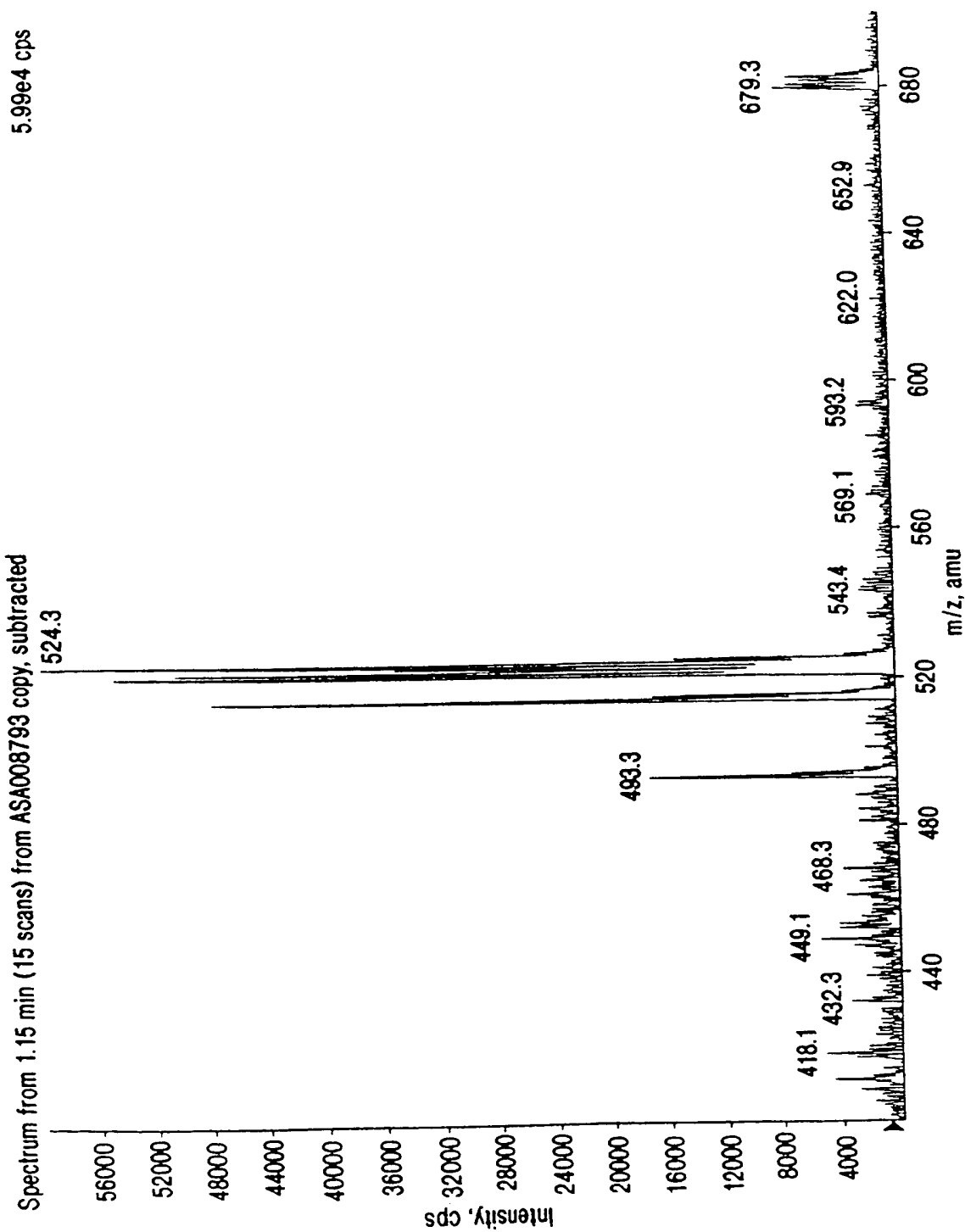


FIG. 264

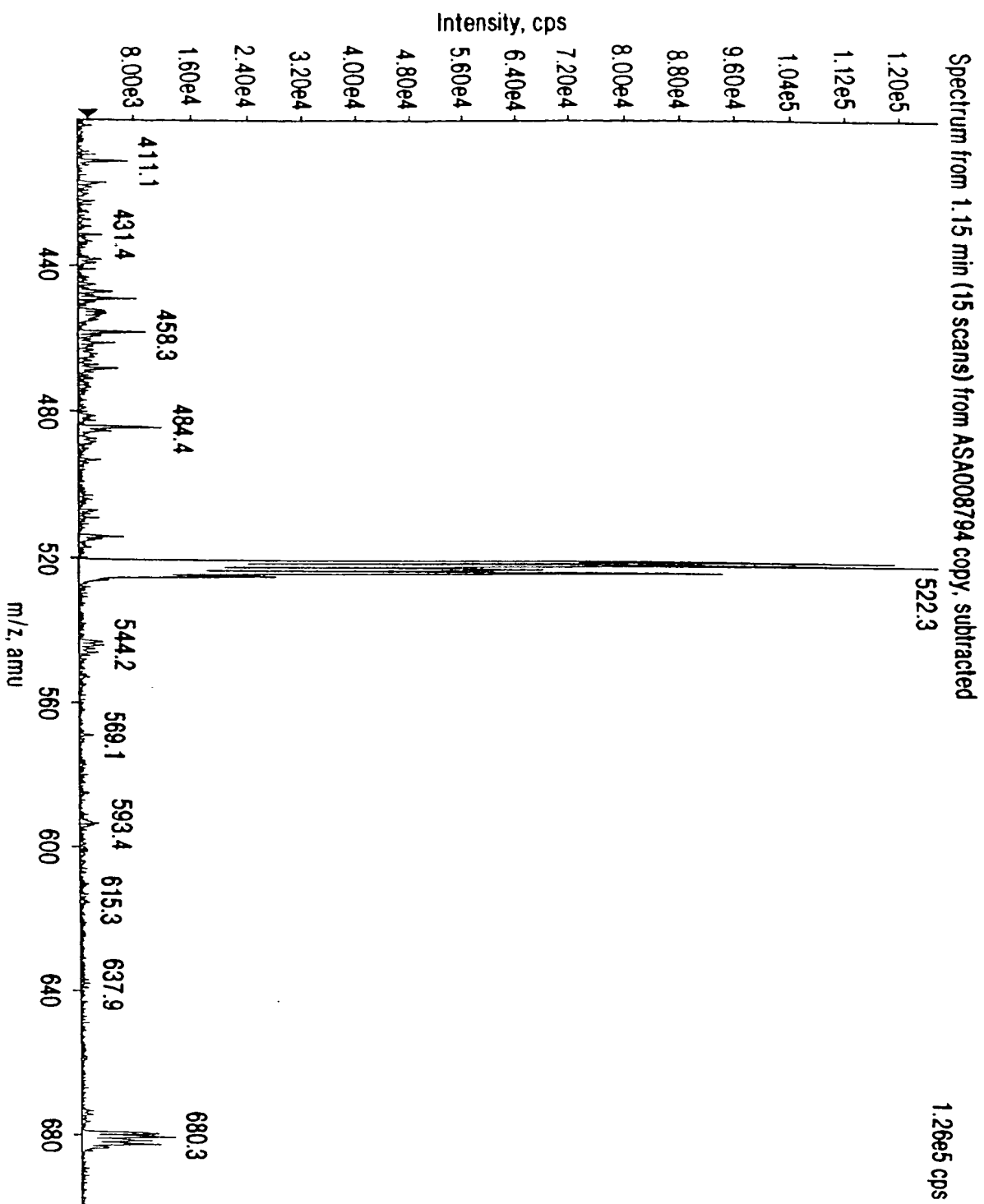


FIG. 265

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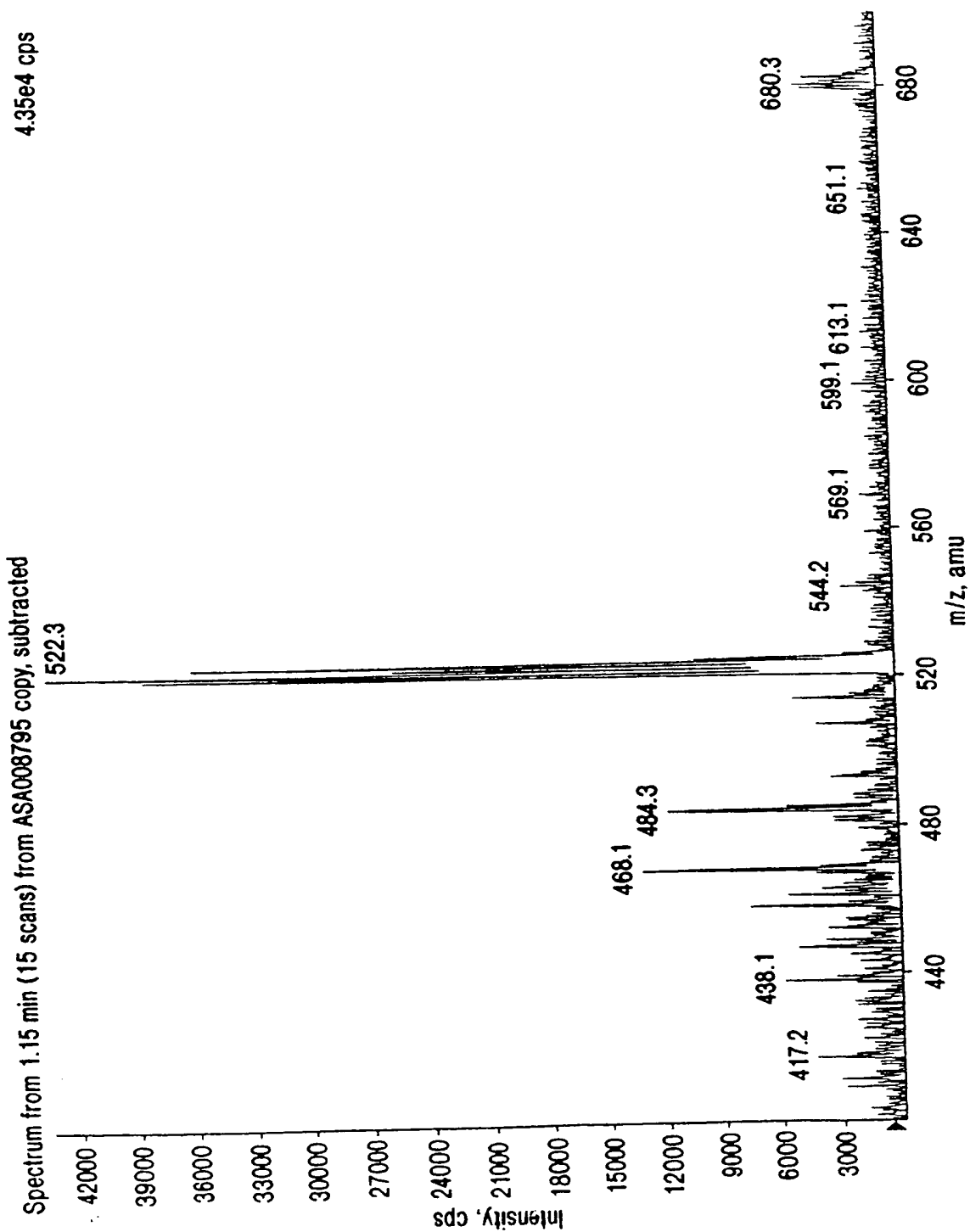


FIG. 266

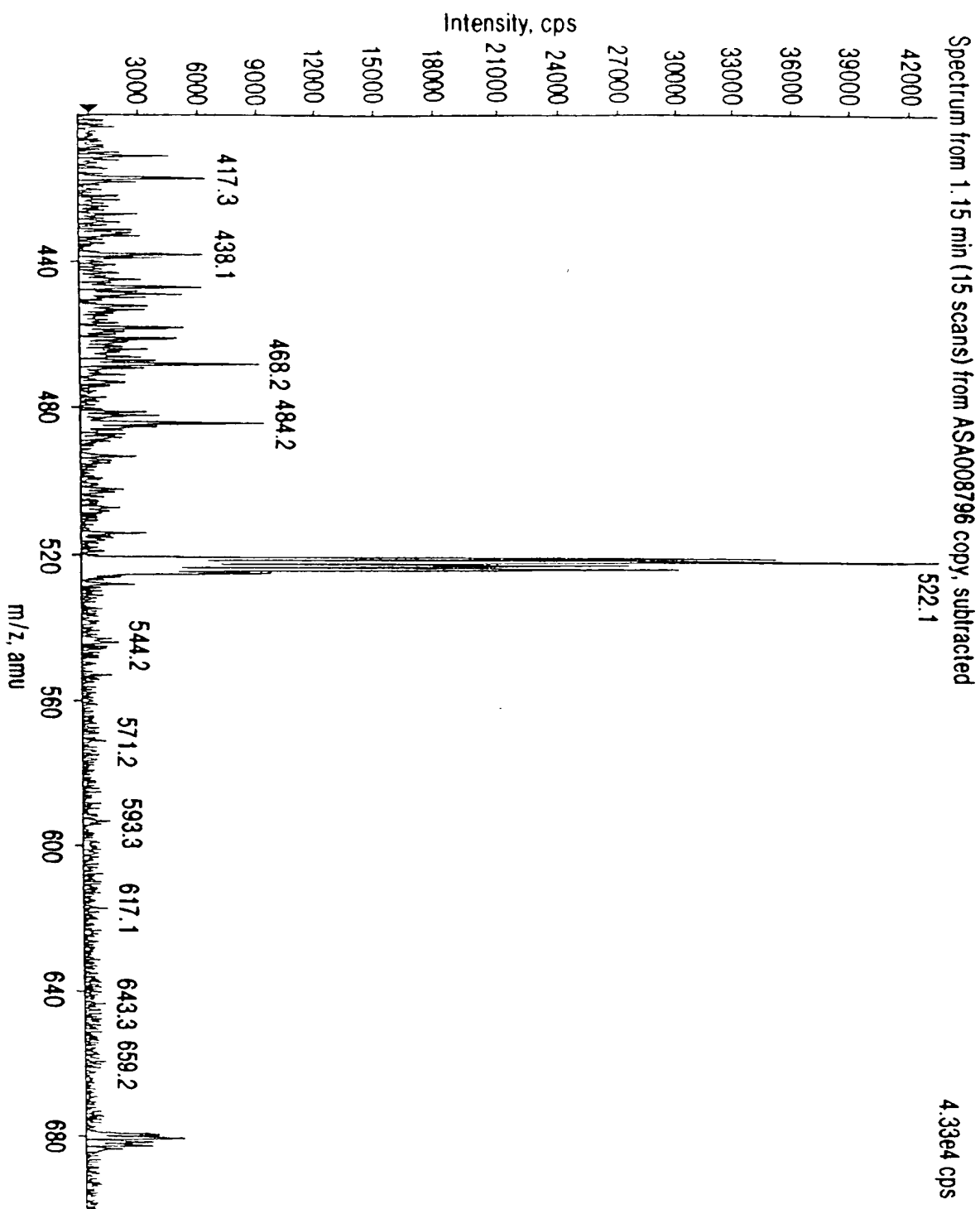


FIG. 267

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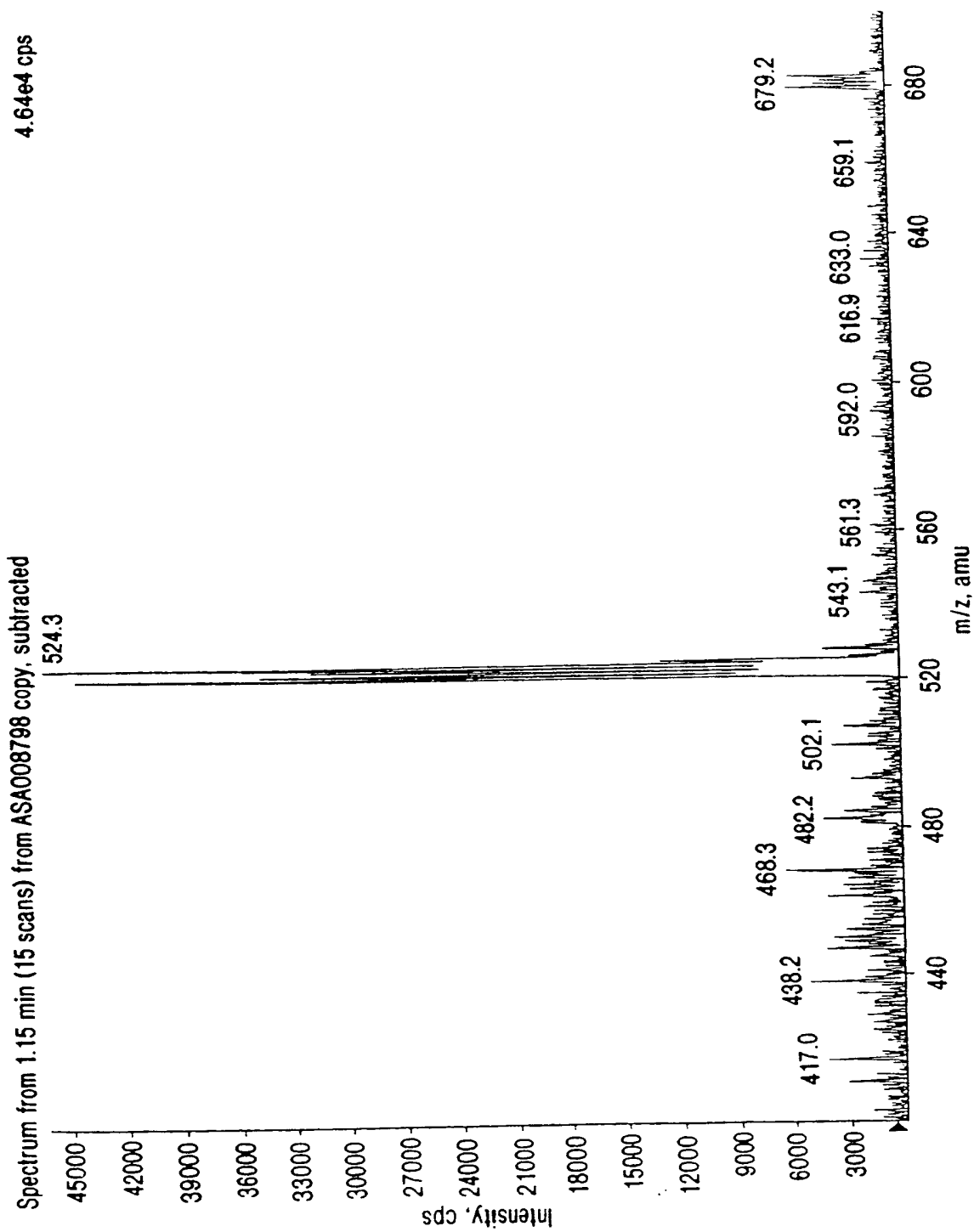


FIG. 268

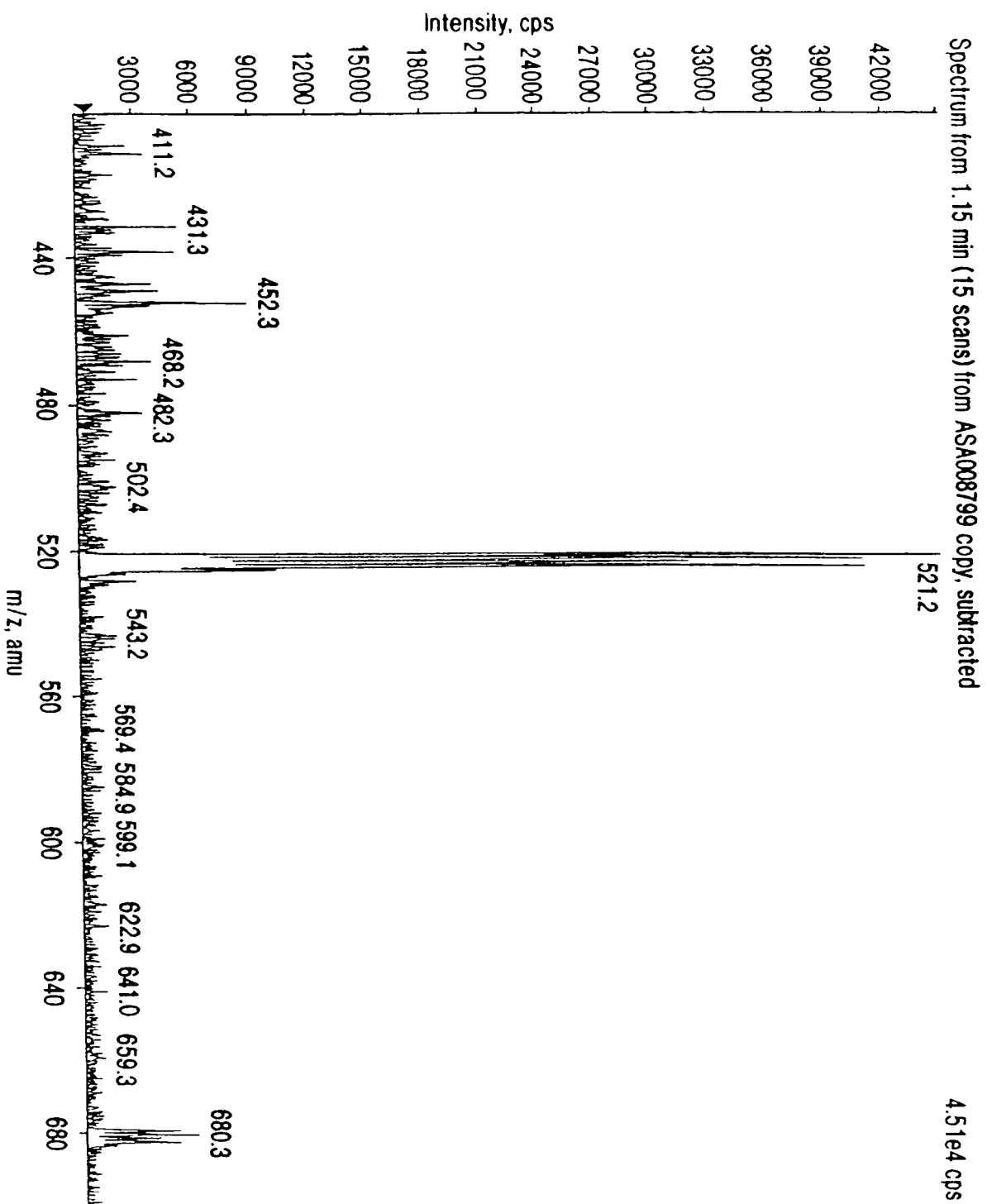


FIG. 269

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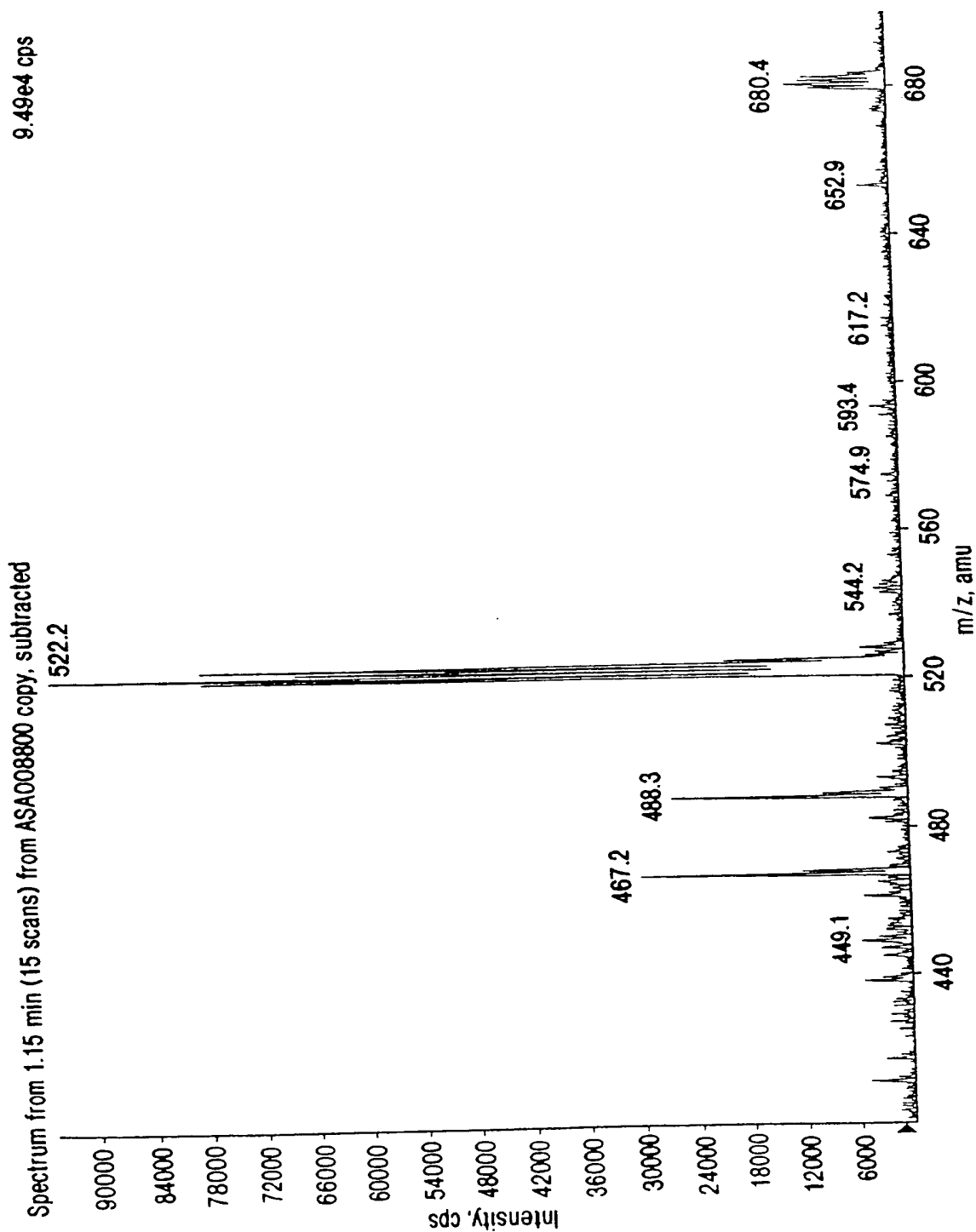


FIG. 270

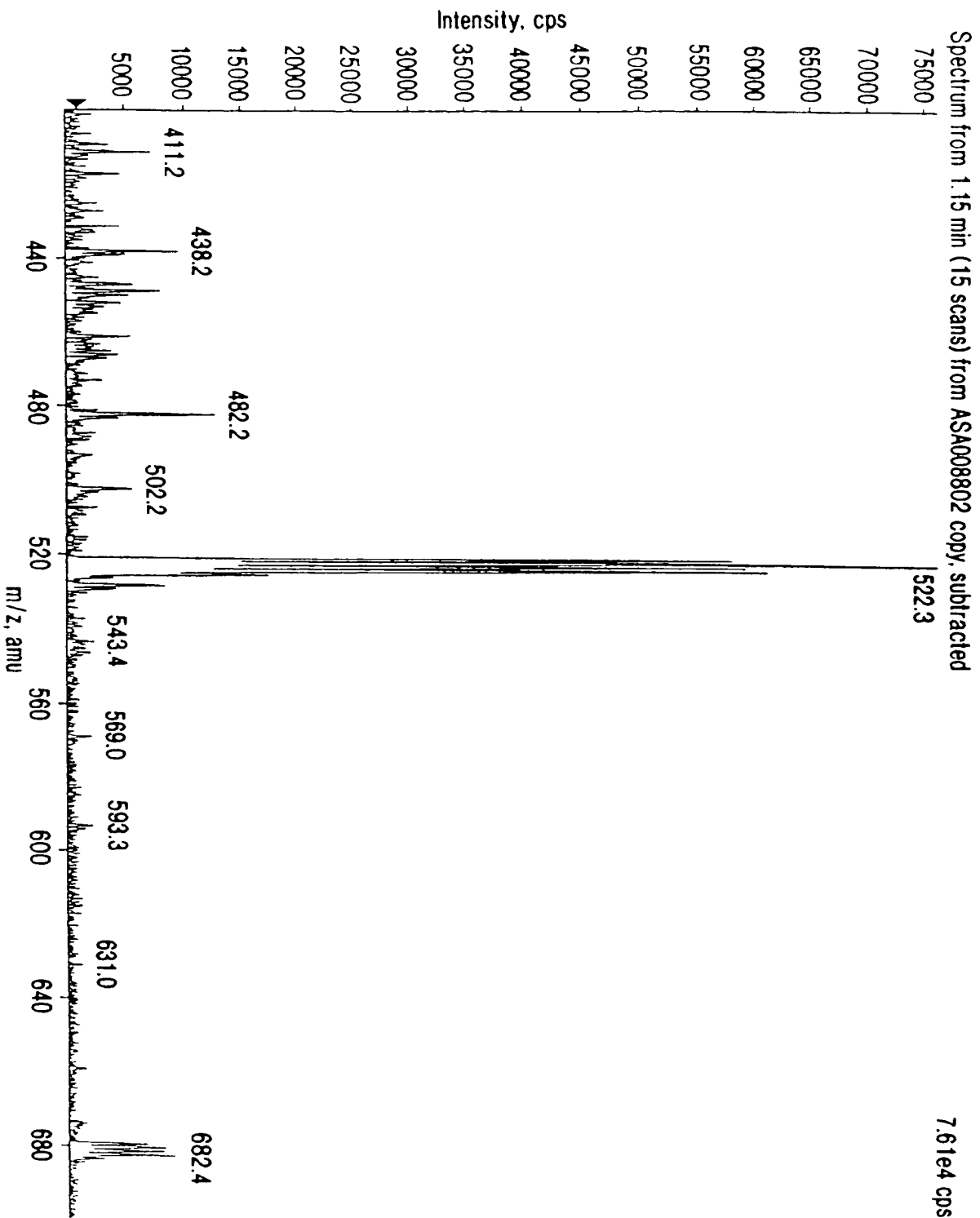


FIG. 271

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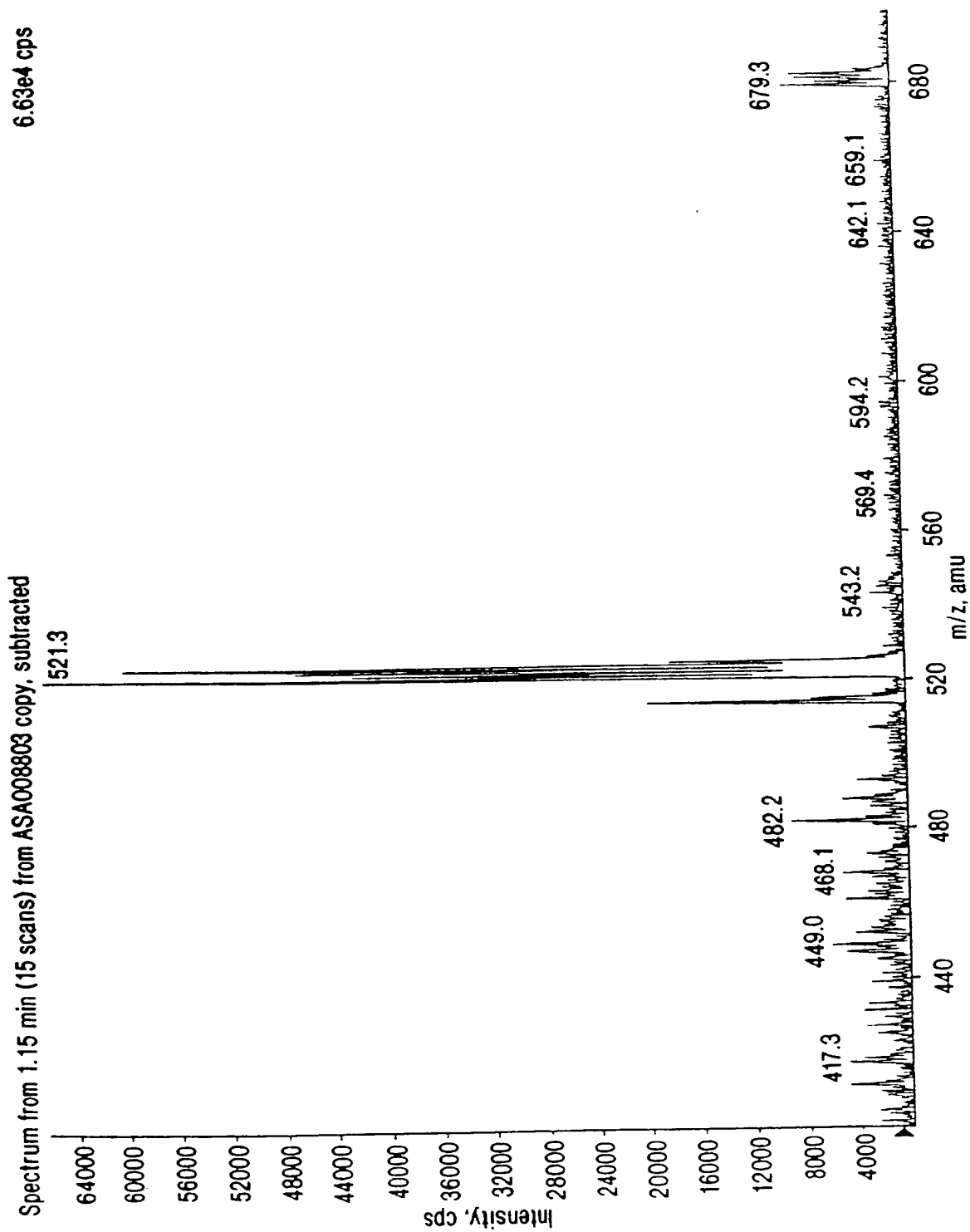


FIG. 272

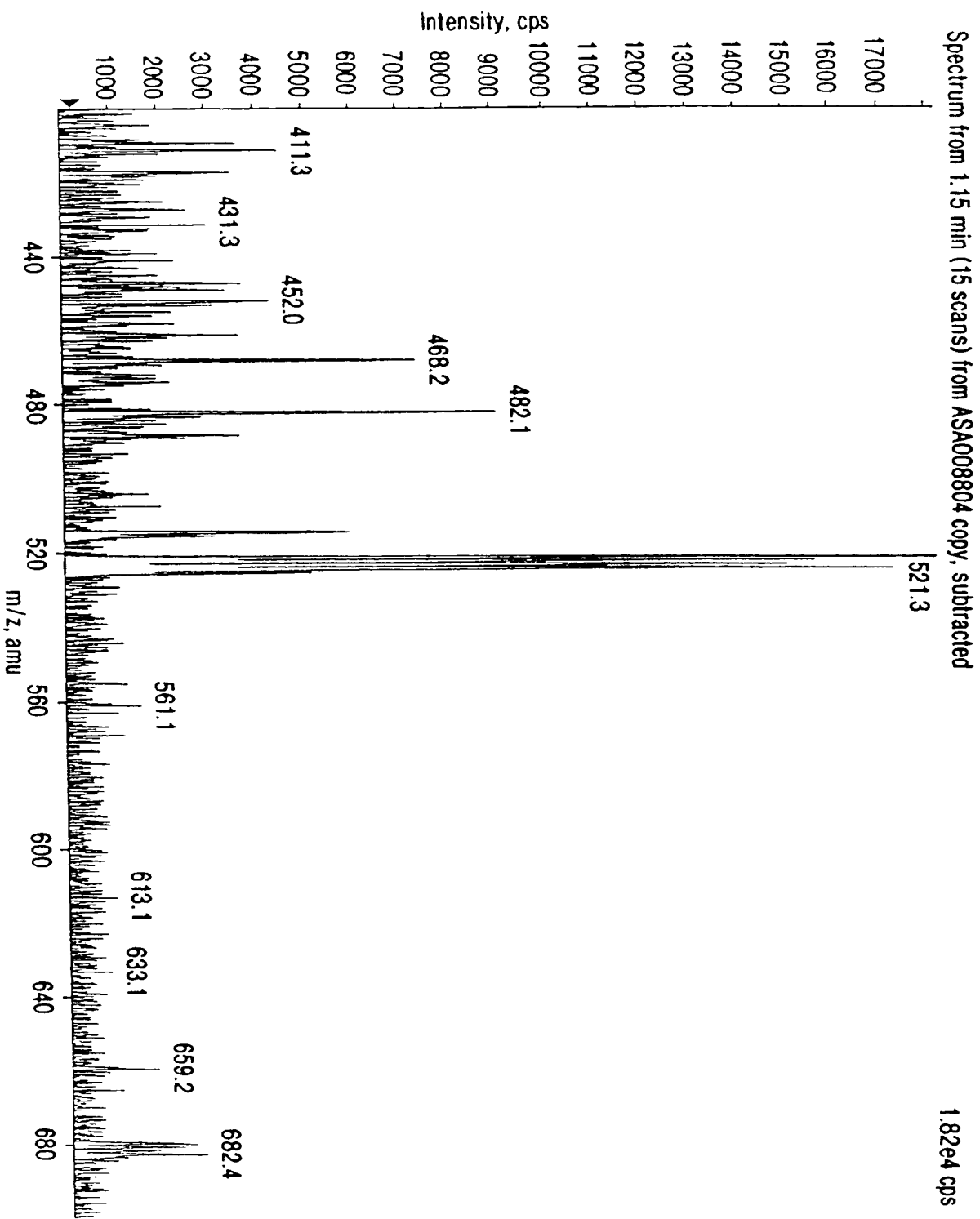


FIG. 273

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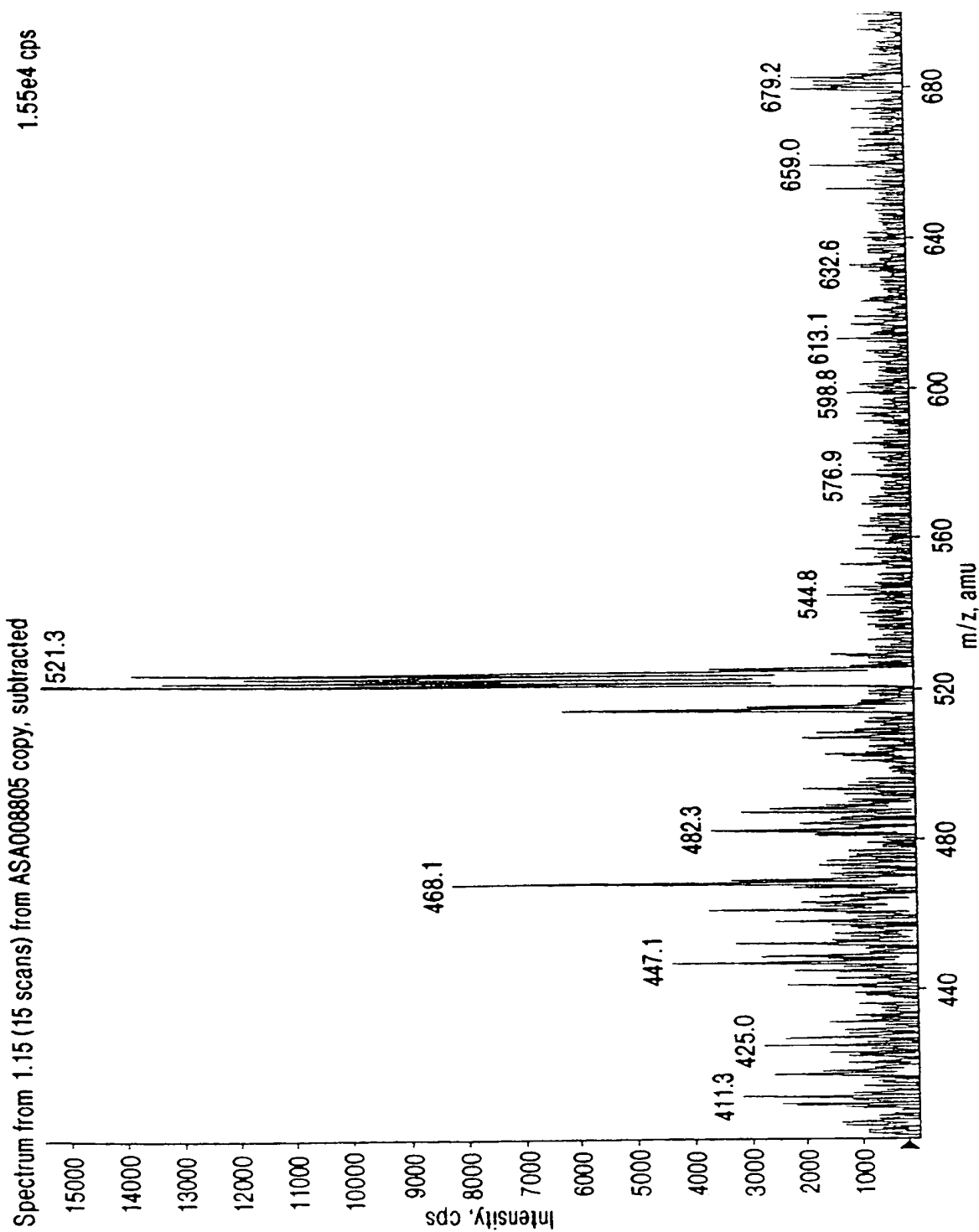


FIG. 274

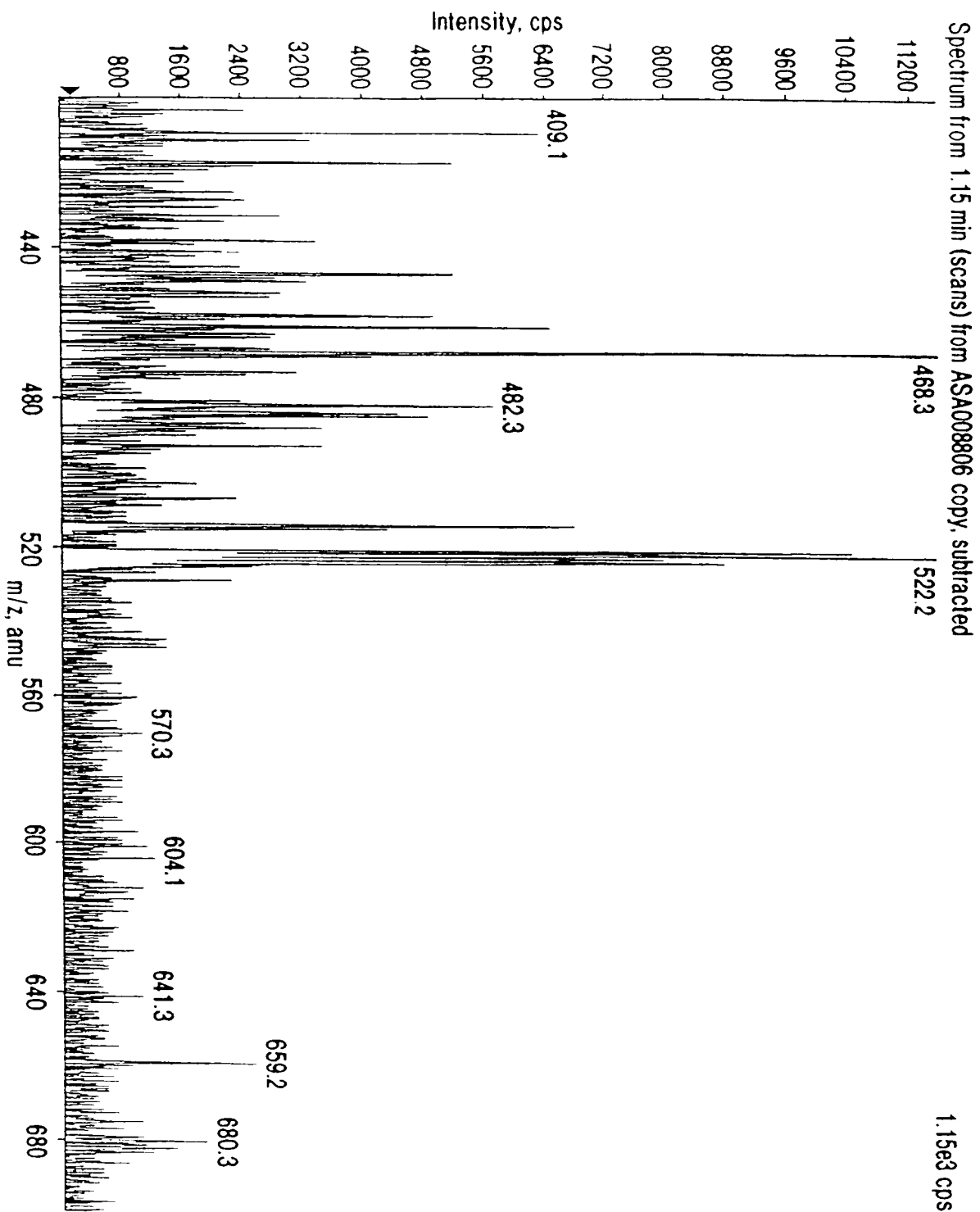


FIG. 275

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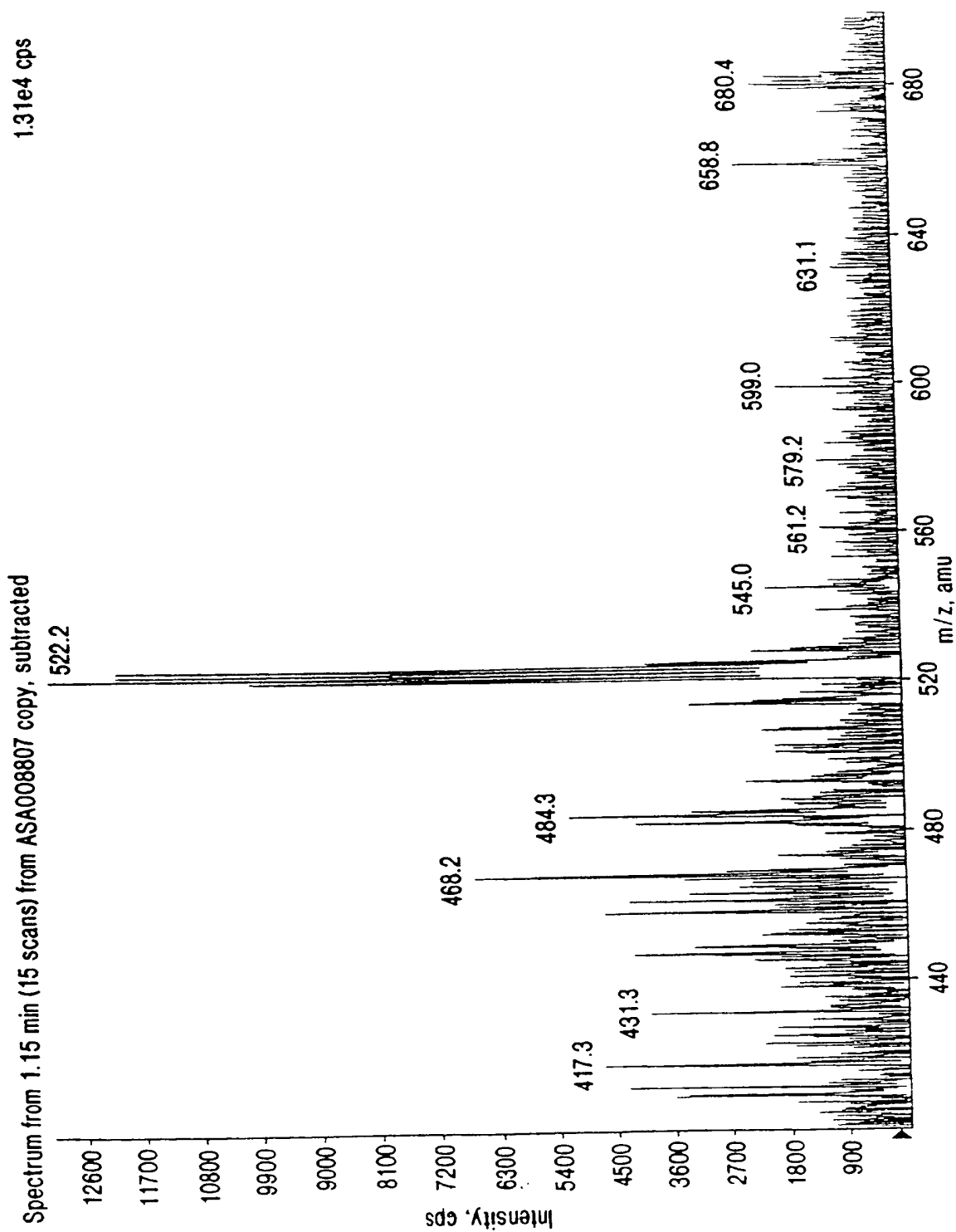


FIG. 276

Model	Count (Series 1)
430.89	~210,000
522.16	~140,000
524.18	~100,000
529.79	~20,000
533.81	~10,000
537.86	~5,000
544.12	~5,000
561.79	~5,000
566.9	~5,000
615.12	~5,000
680.2	~10,000
682.2	~10,000

FIG. 277

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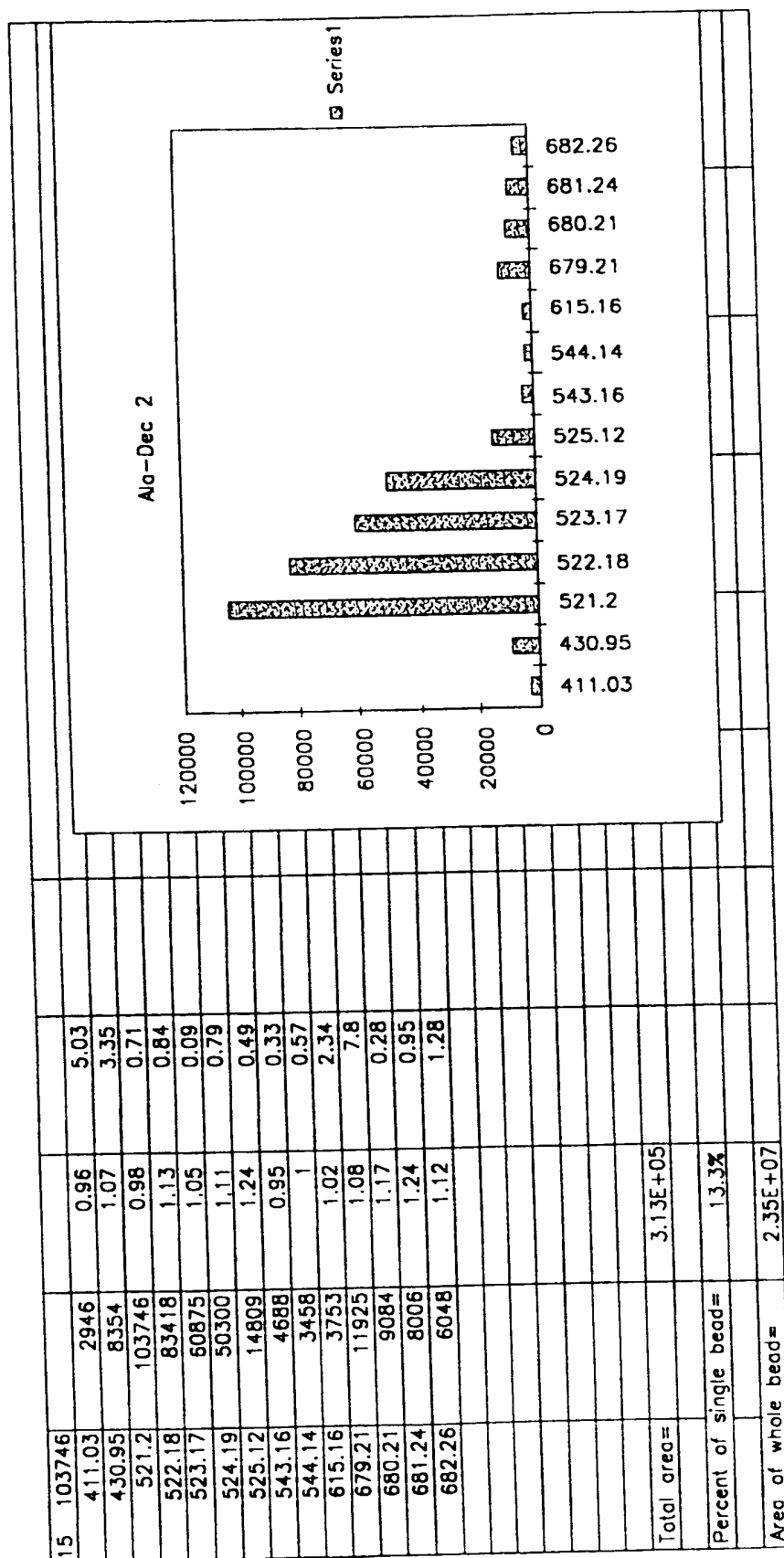
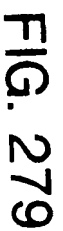
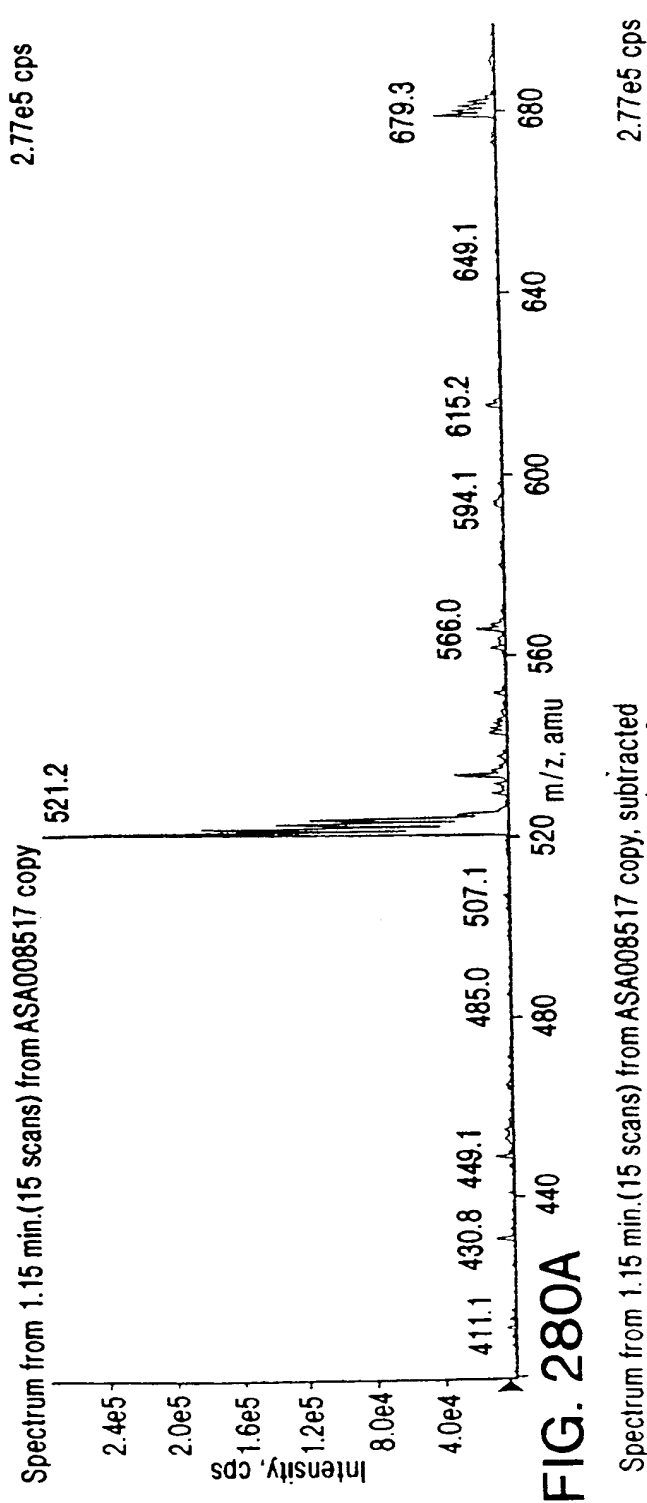


FIG. 278



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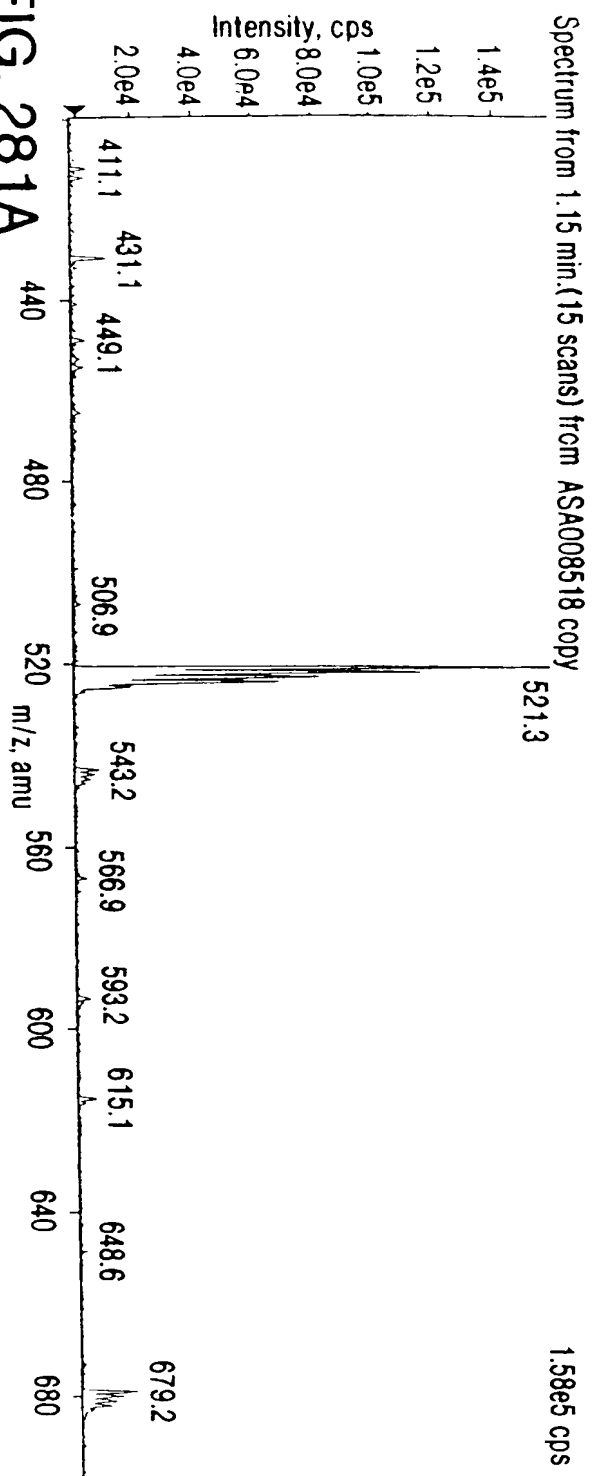


FIG. 281A

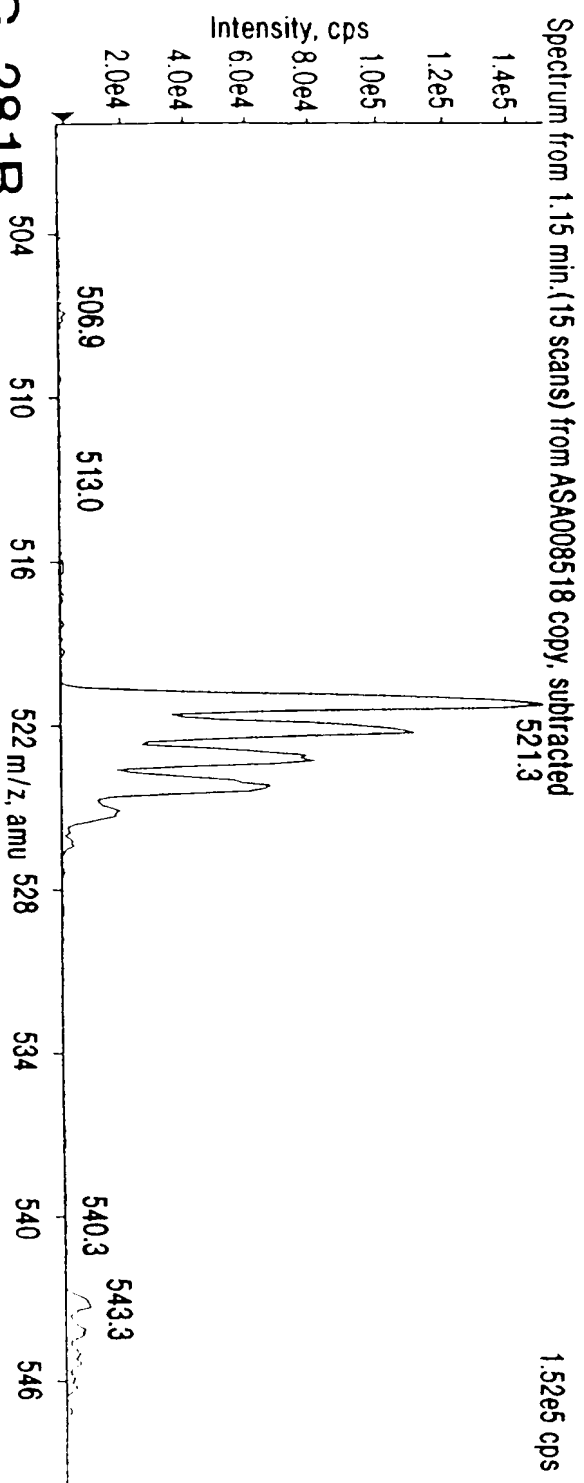
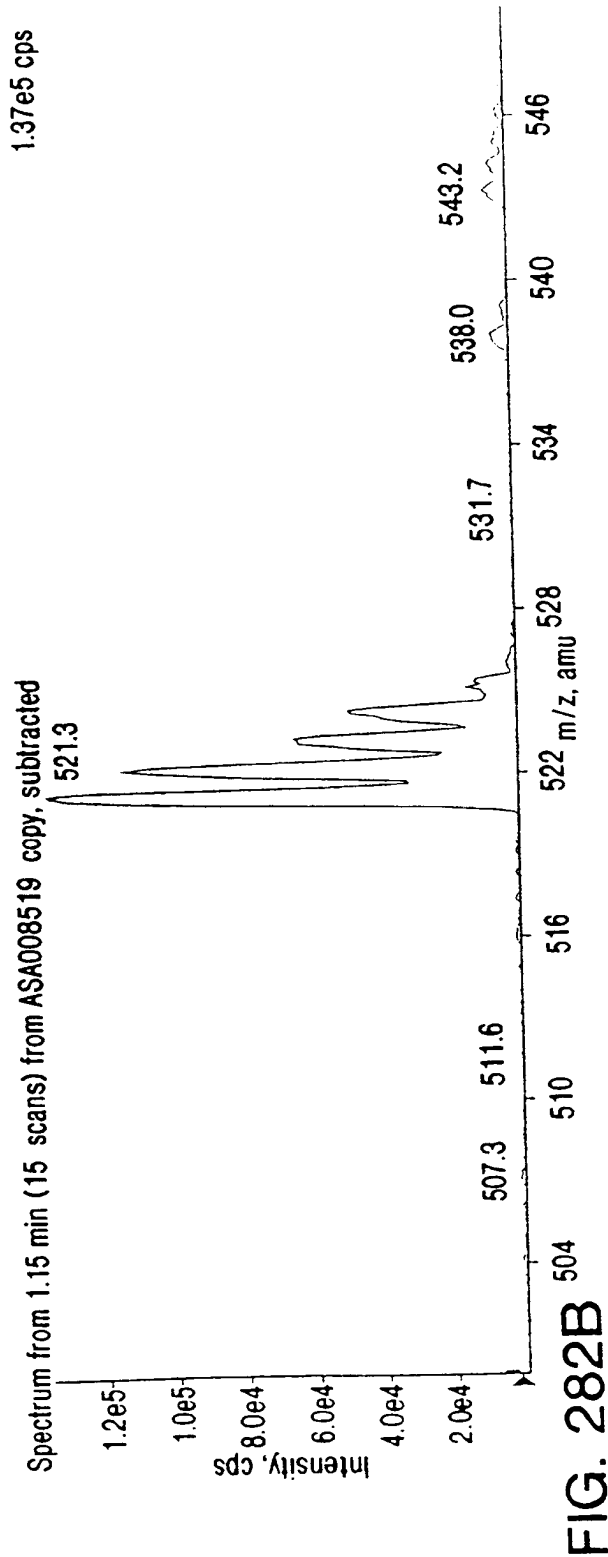
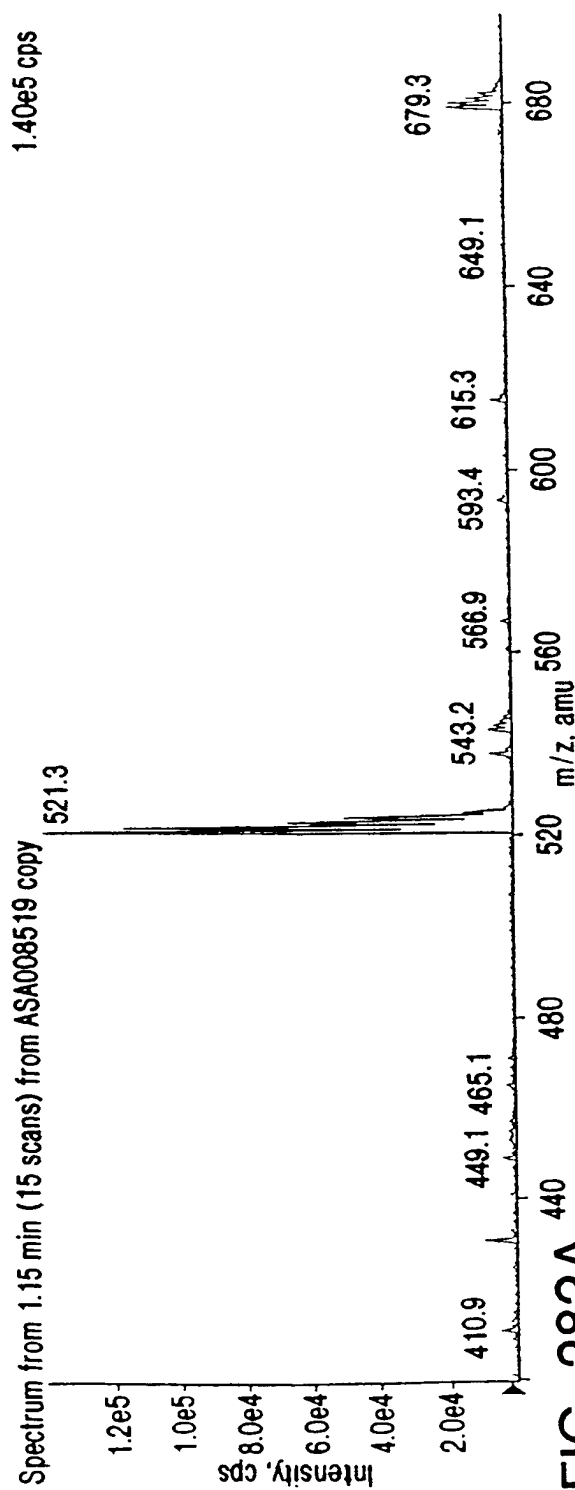
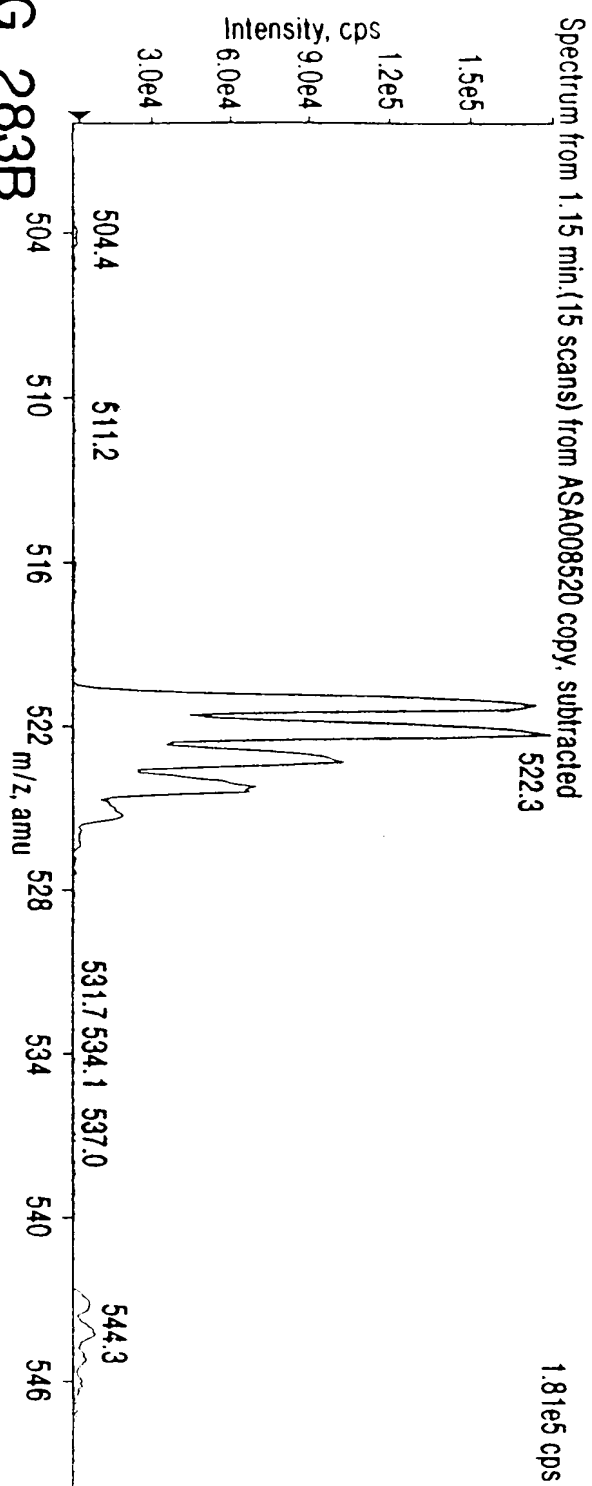
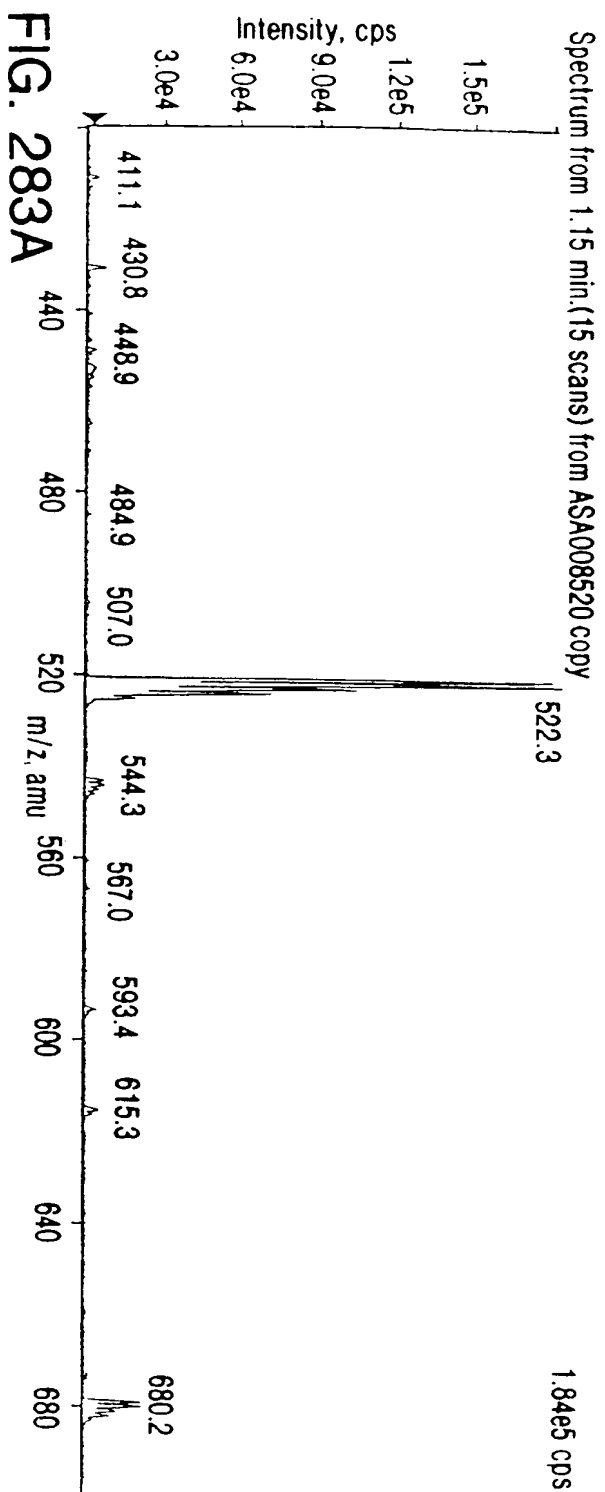


FIG. 281B

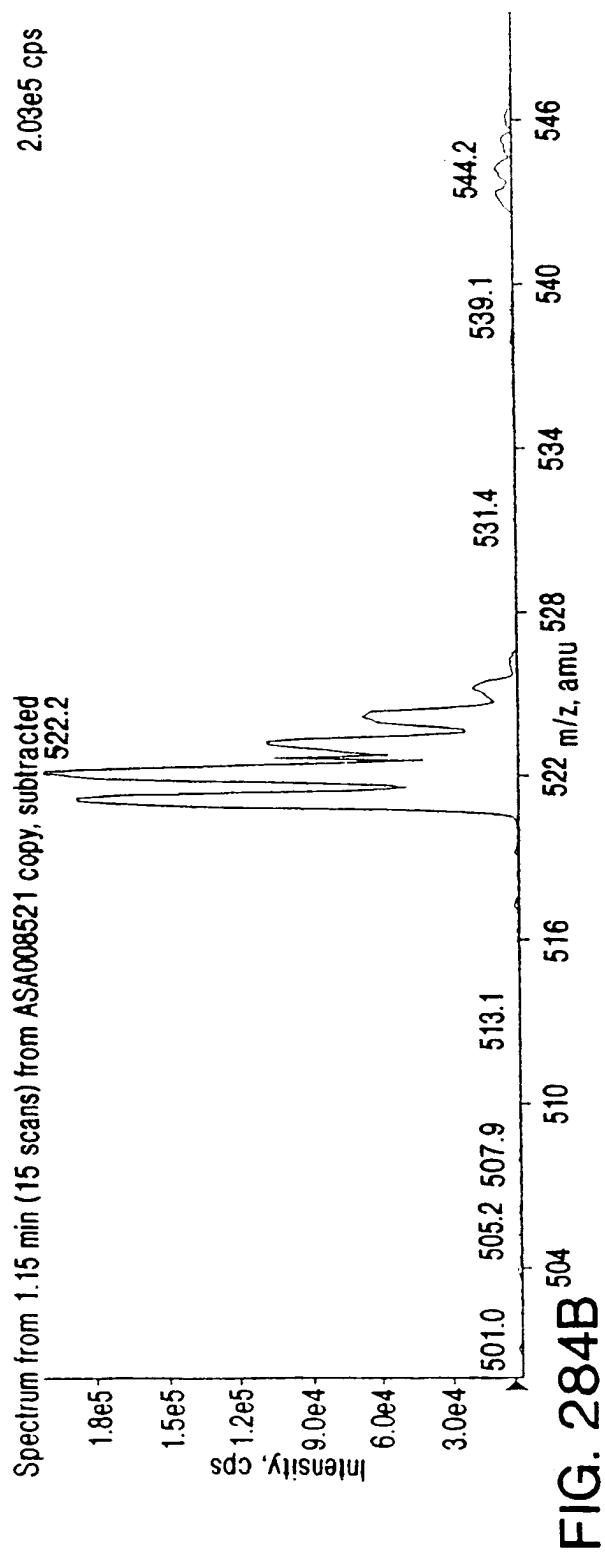
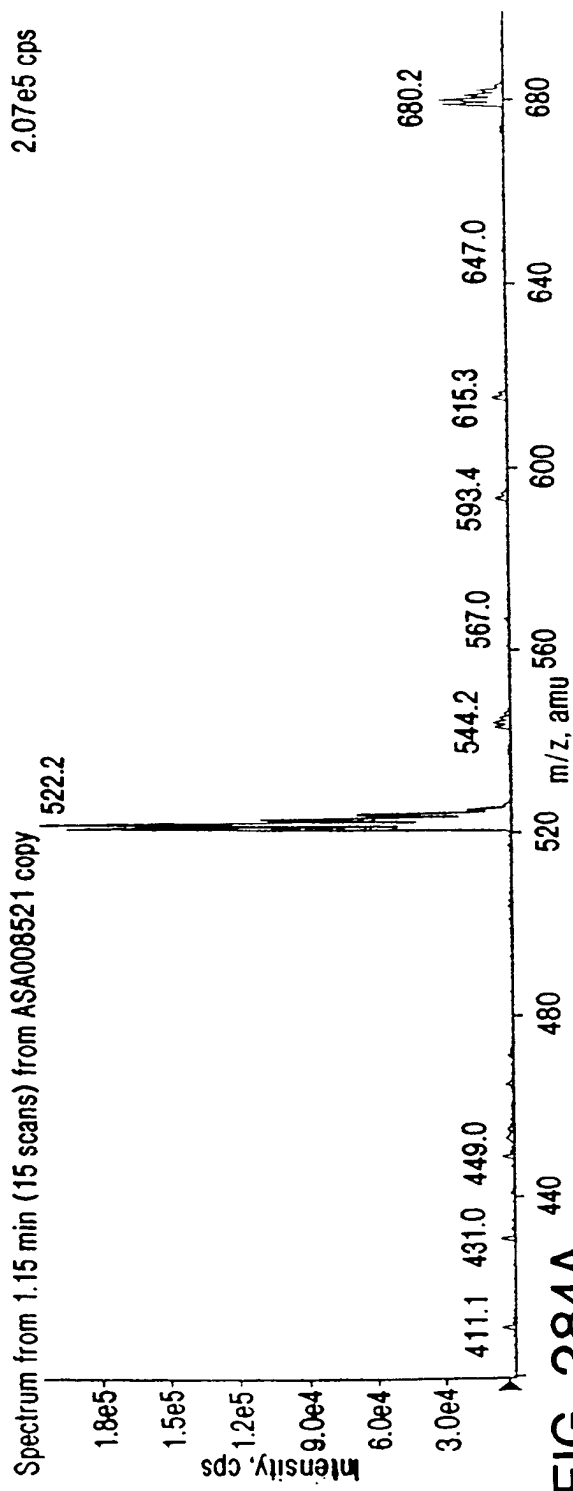
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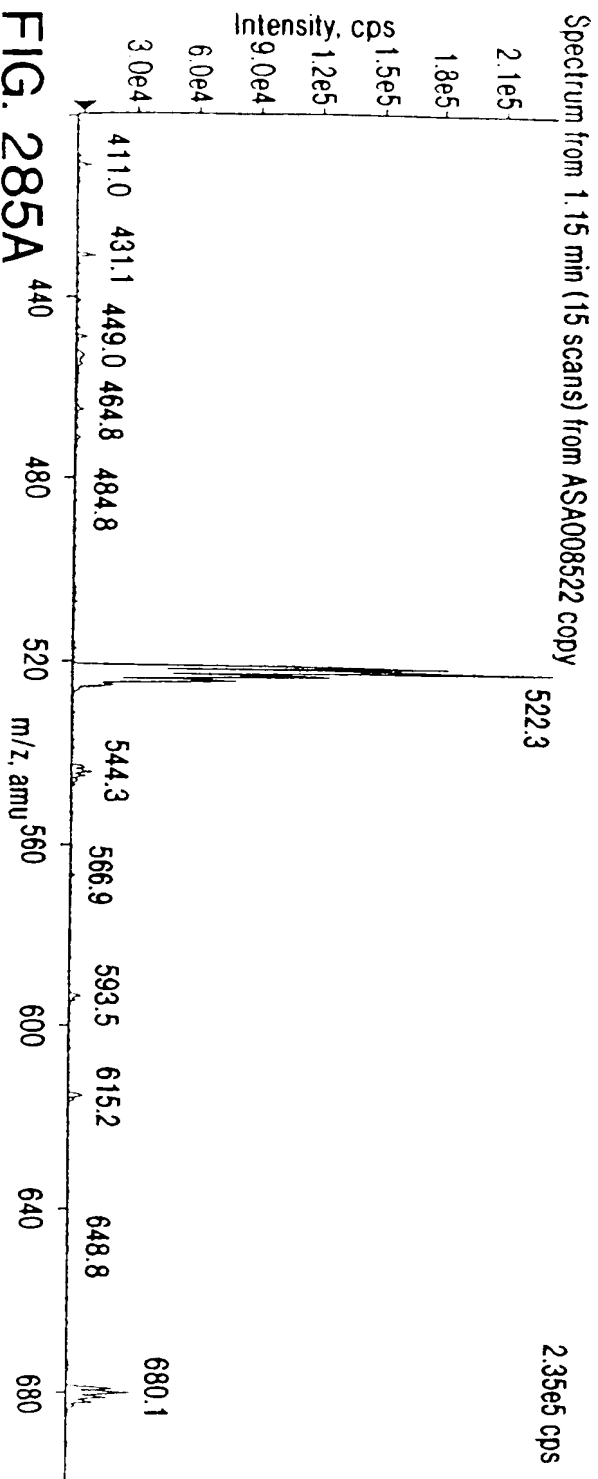


FIG. 285A

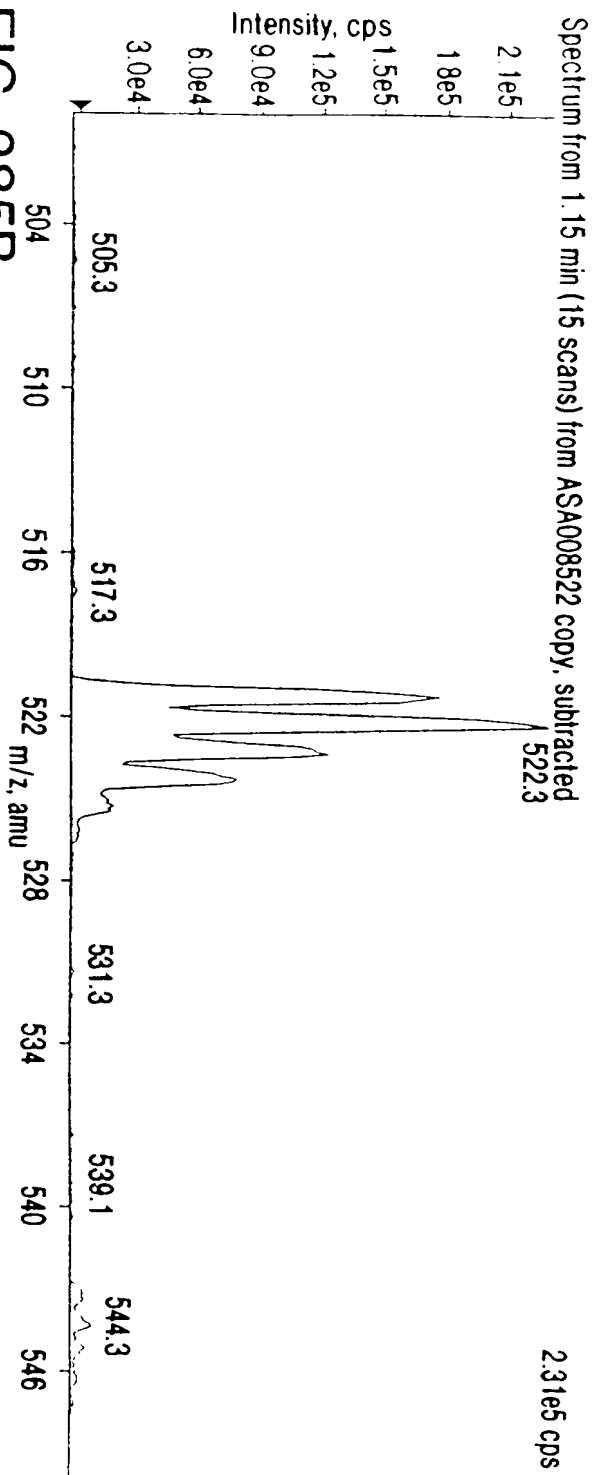


FIG. 285B

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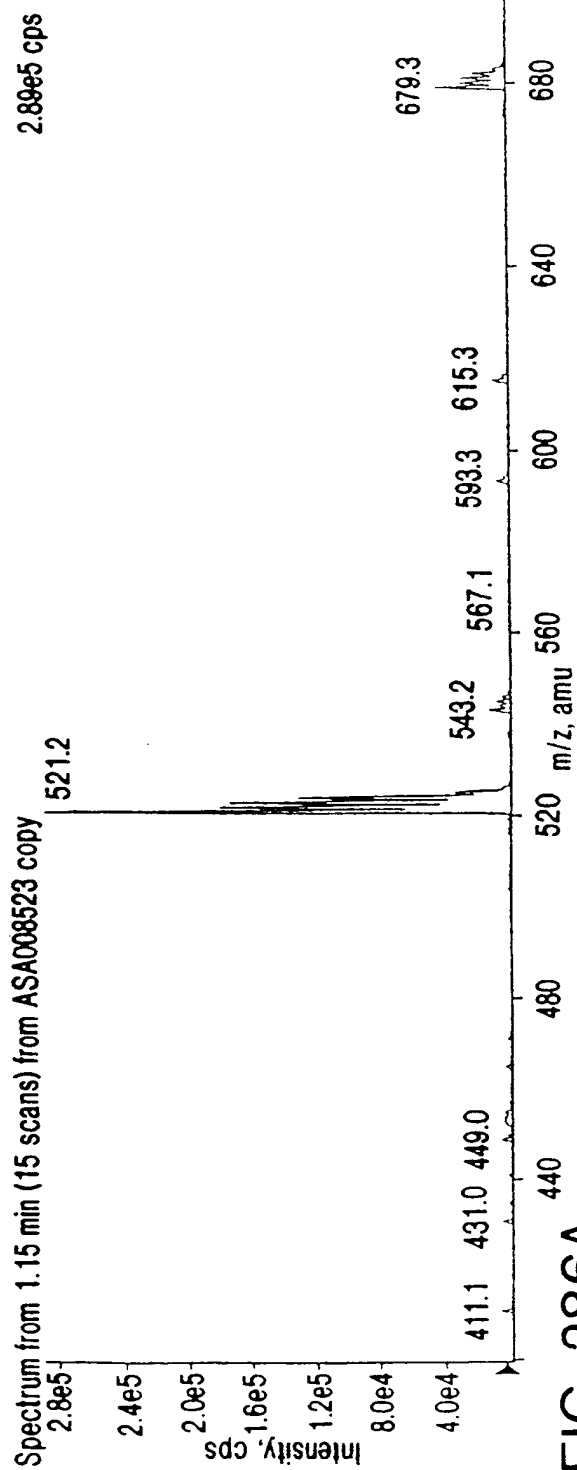


FIG. 286A

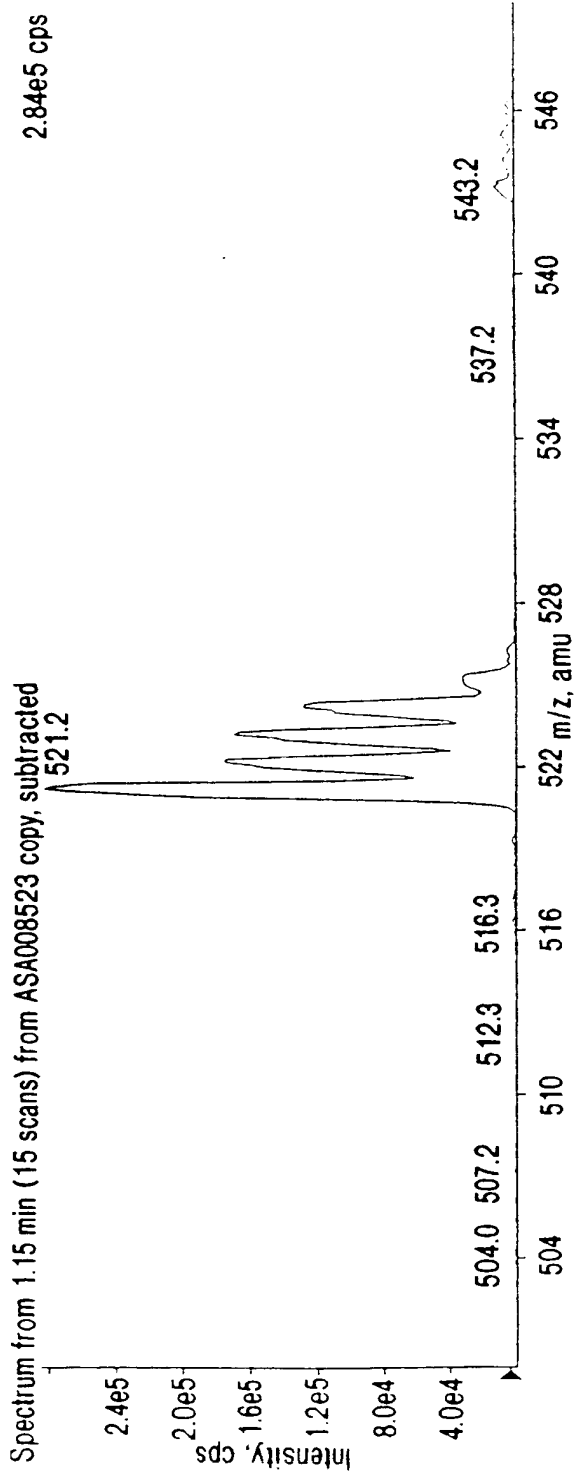
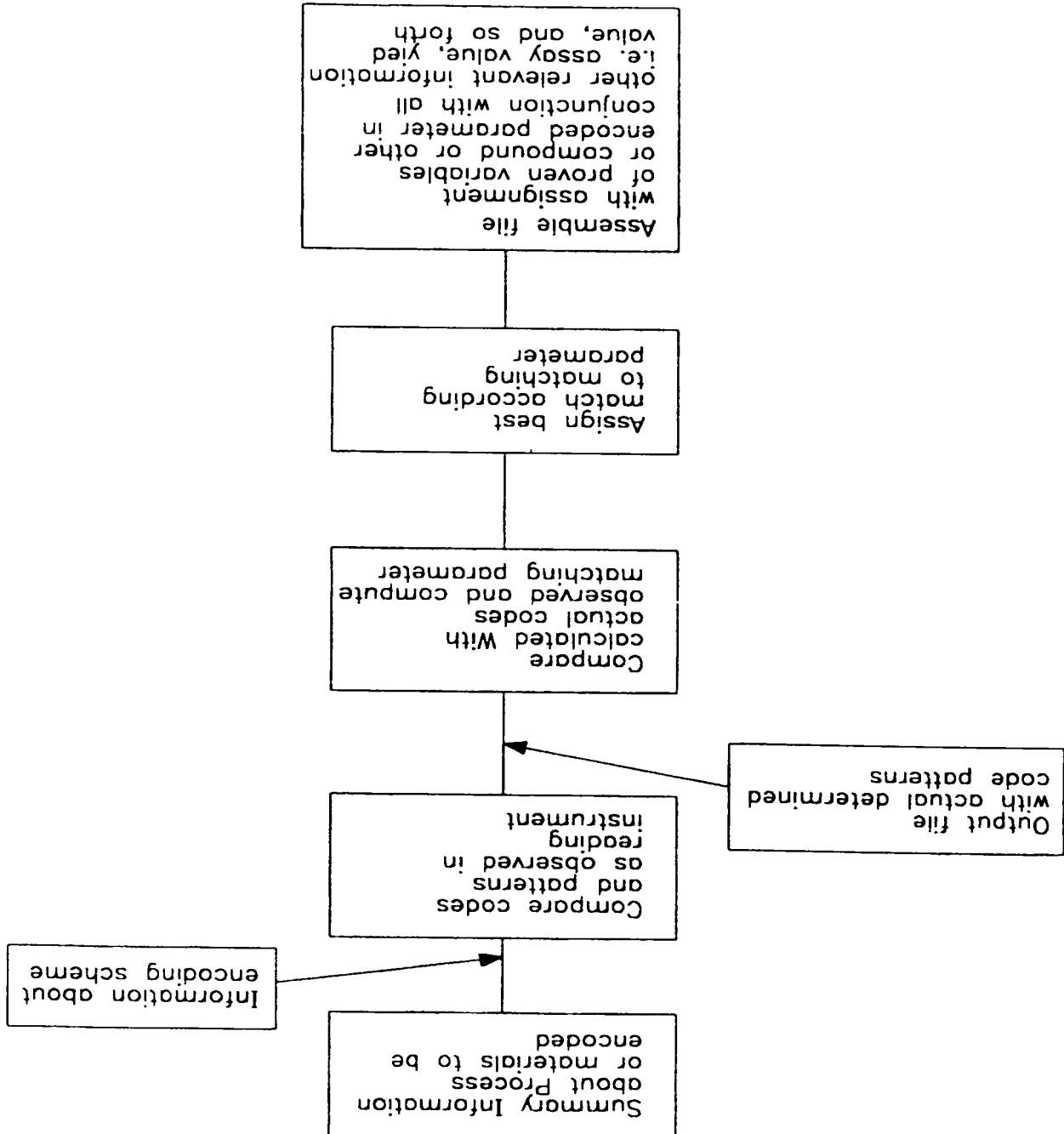


FIG. 286B

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FIG. 287



INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US 97/05701

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07B61/00 C07K1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07B C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 28640 A (TRUSTEES OF COLUMBIA UNIVERSITY) 26 October 1995 see claims 1-11; page 53, line 15 to page 57, line 9; page 58, line 6 to page 59, line 29; page 64, line 31 to page 66, line 4; examples 2 and 10; pages 135-138 ---	1-11, 20-34, 47, 54-56, 62-72, 77,78
P,X	CHEMISTRY & BIOLOGY, vol. 3, no. 8, August 1996, pages 679-688, XP002035873 H. M. GEYSEN ET AL: "Isotope or mass encoding of combinatorial libraries" see the whole document --- -/--	1-78

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

23 July 1997

Date of mailing of the international search report

05.08.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Wright, M

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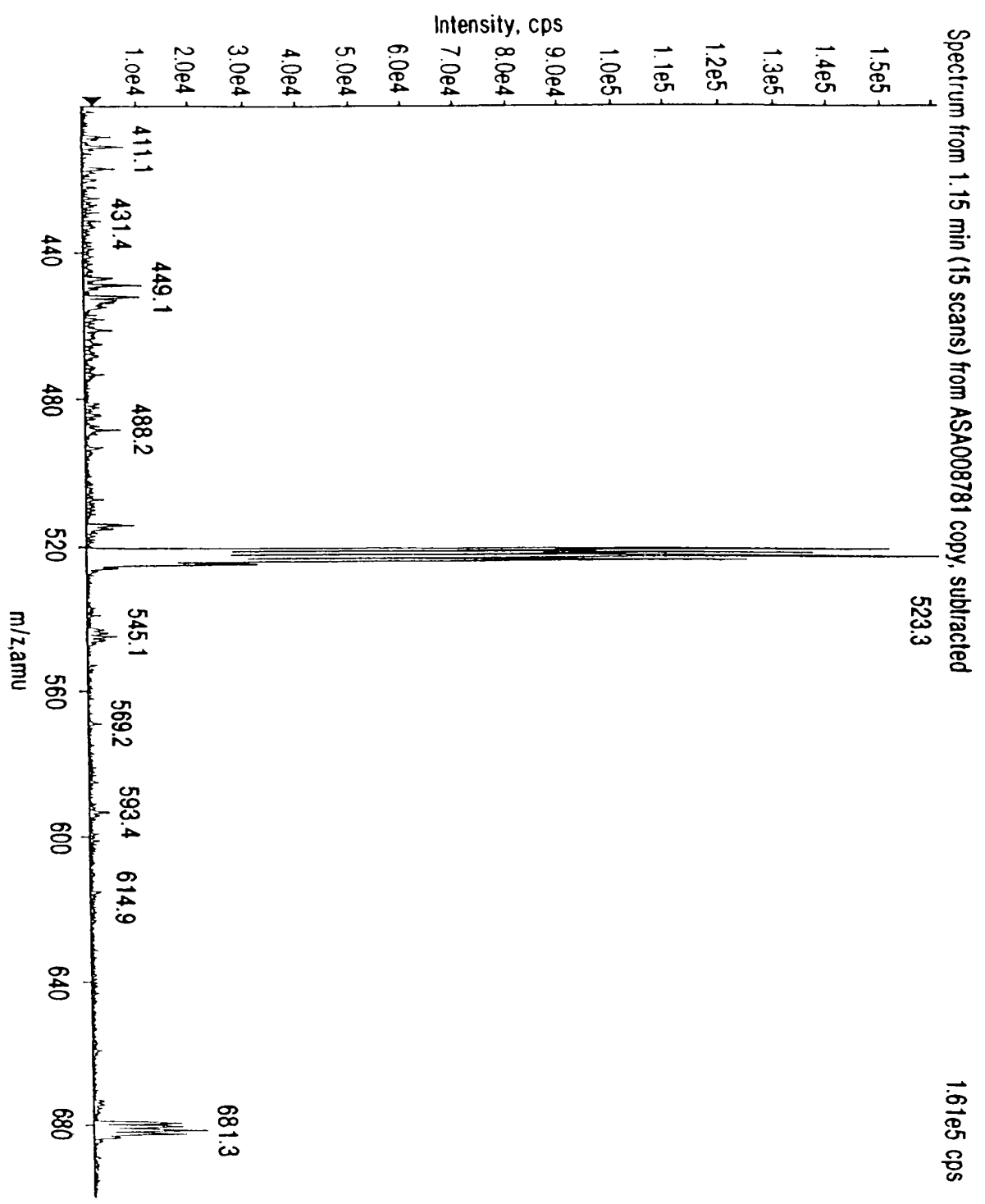


FIG. 255

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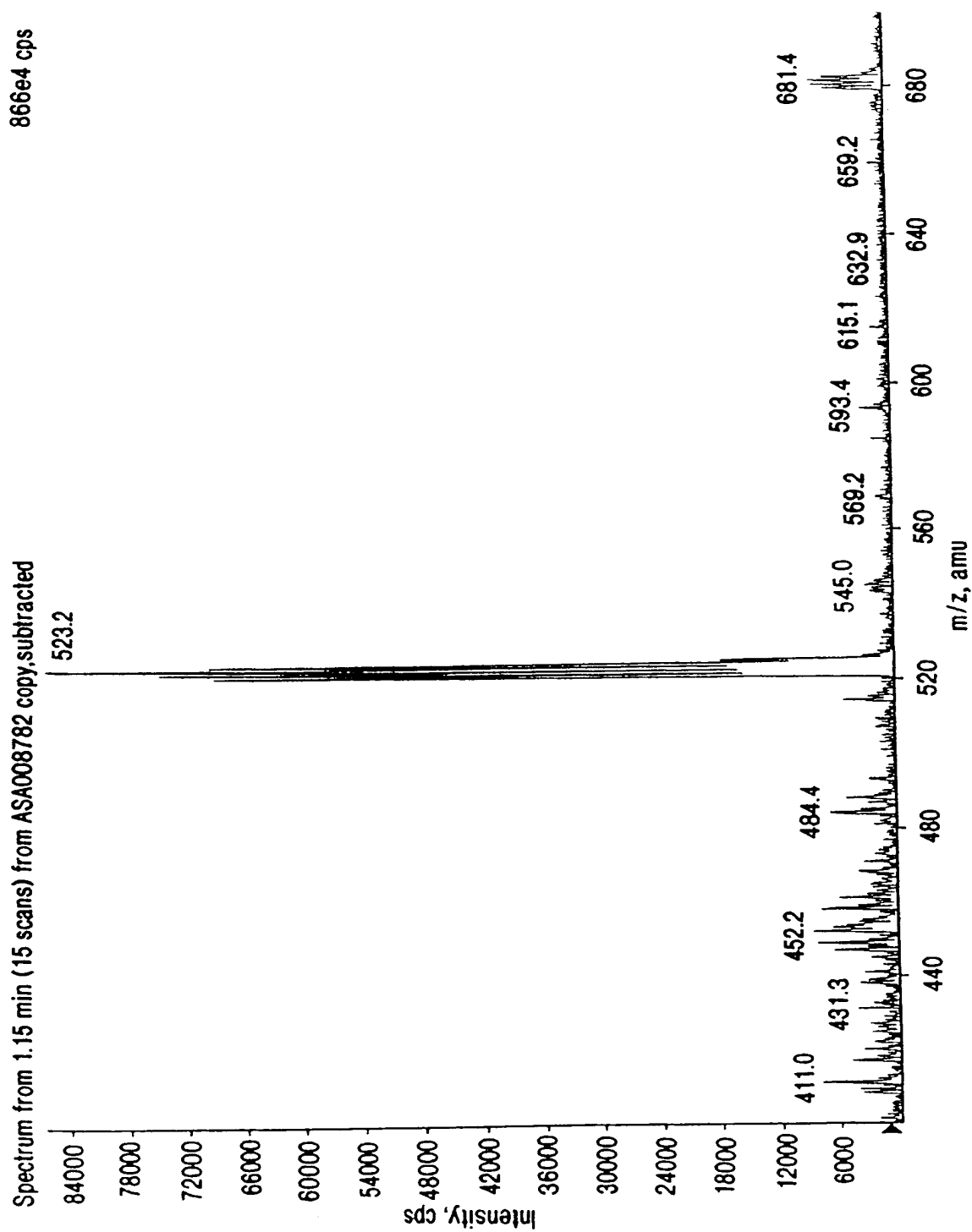


FIG. 256

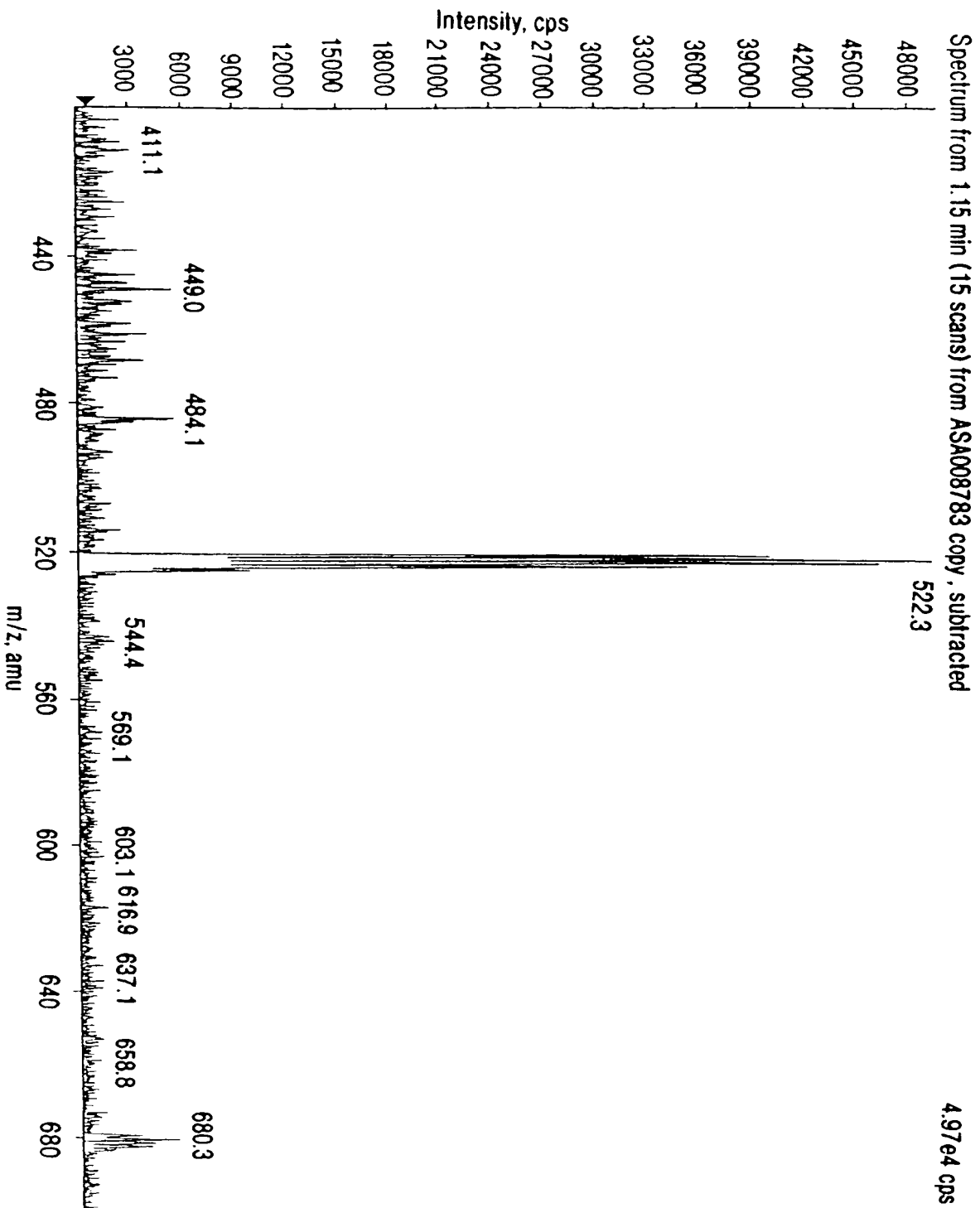


FIG. 257

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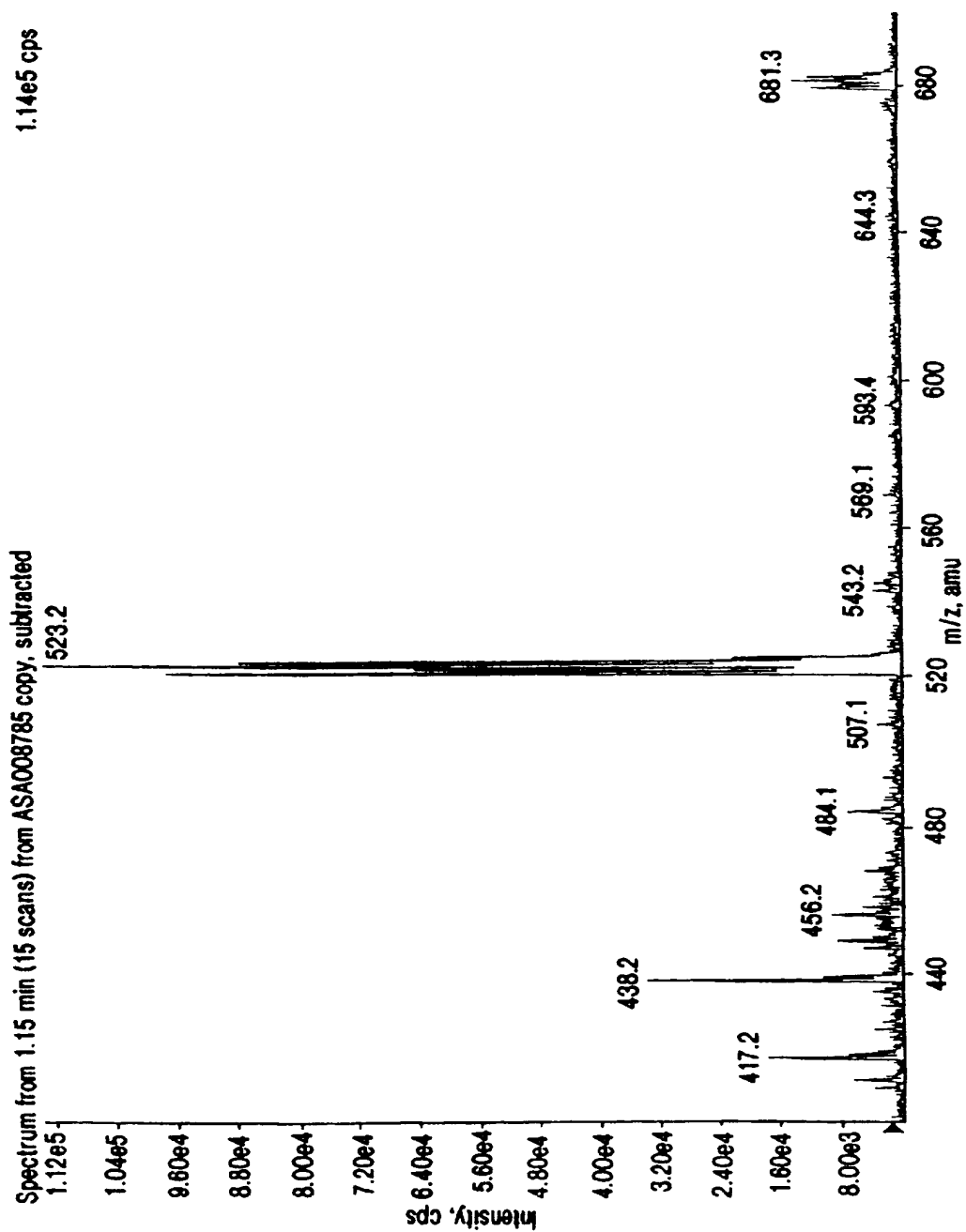


FIG. 258

SUBSTITUTE SHEET (RULE 26)

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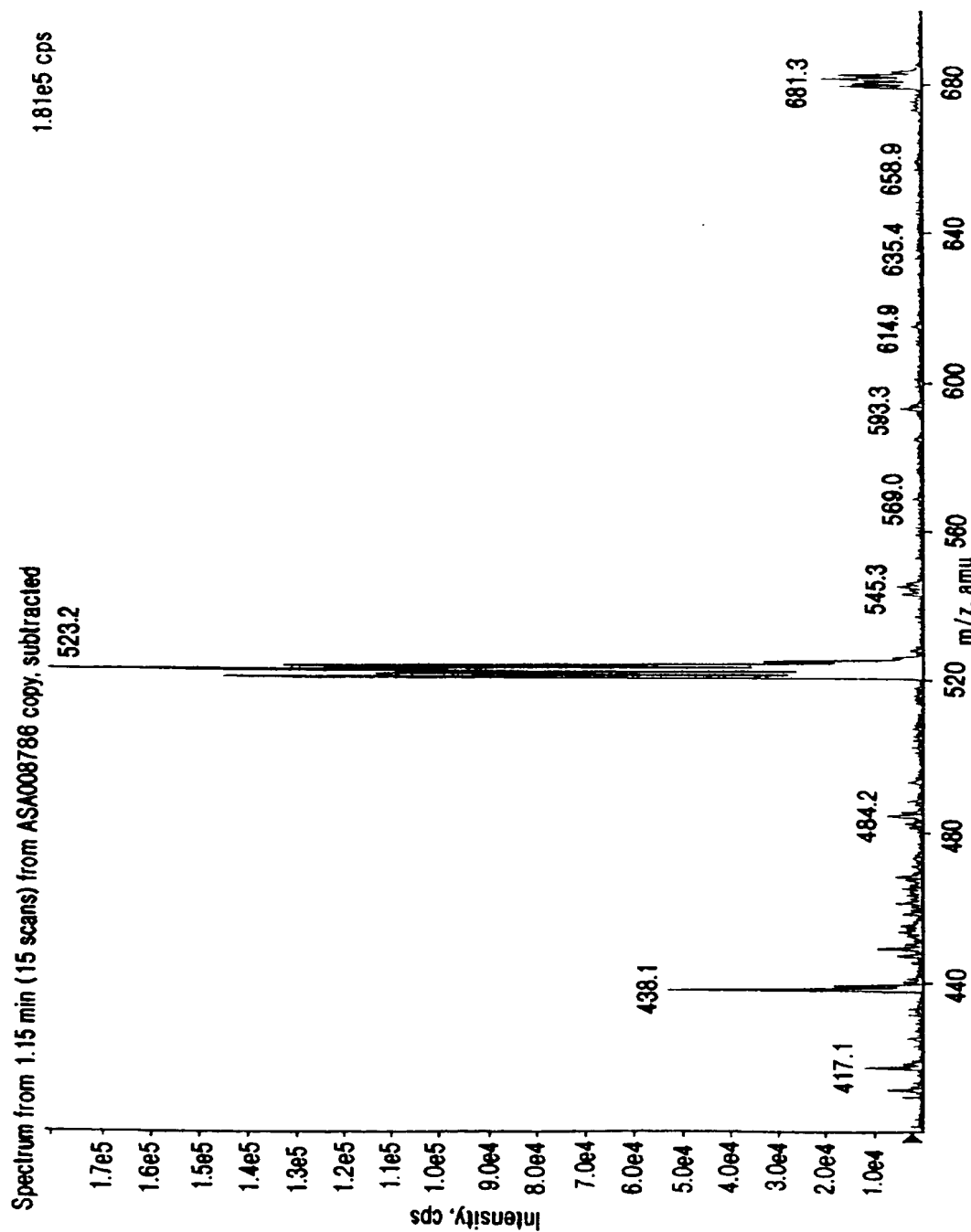


FIG. 259

SUBSTITUTE SHEET (RULE 26)

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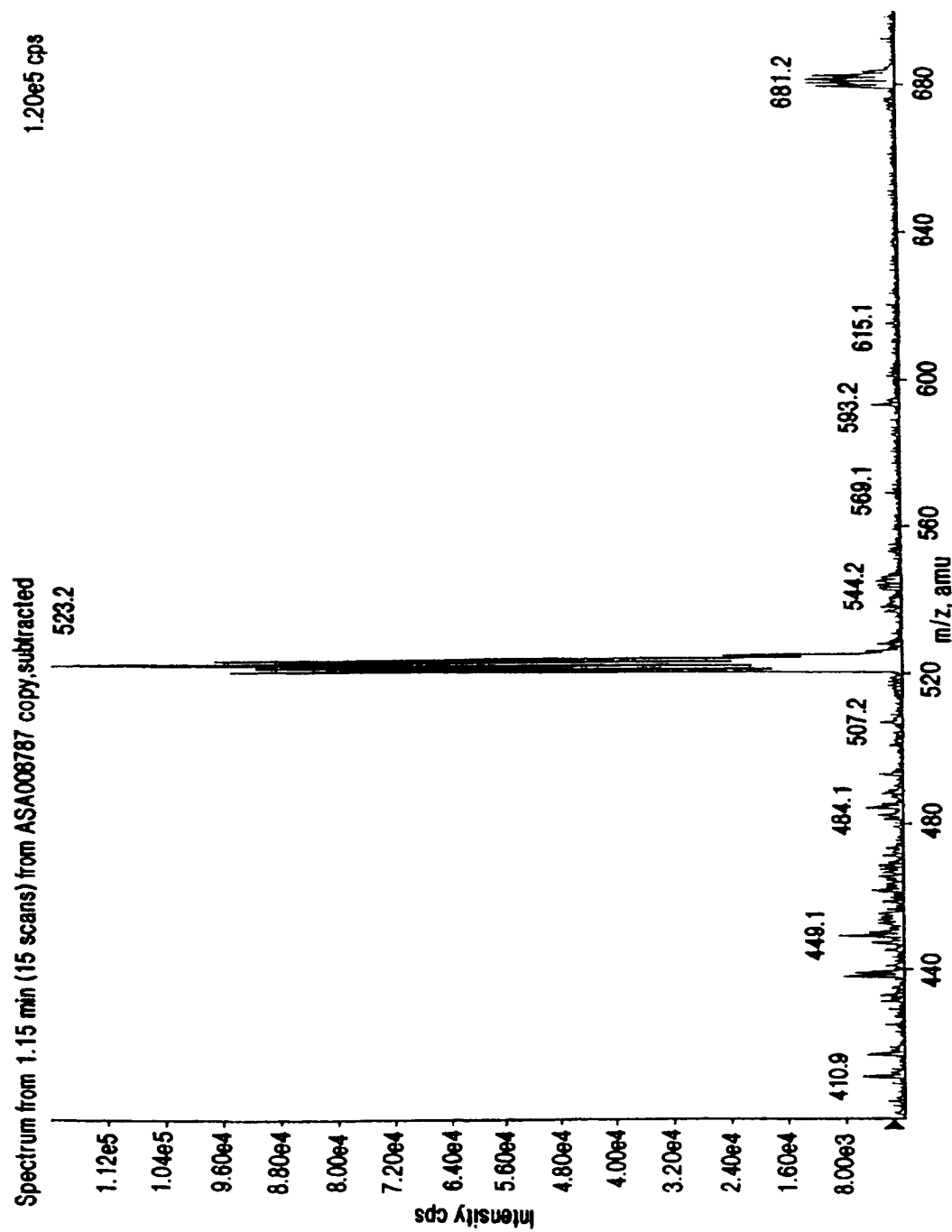


FIG. 260

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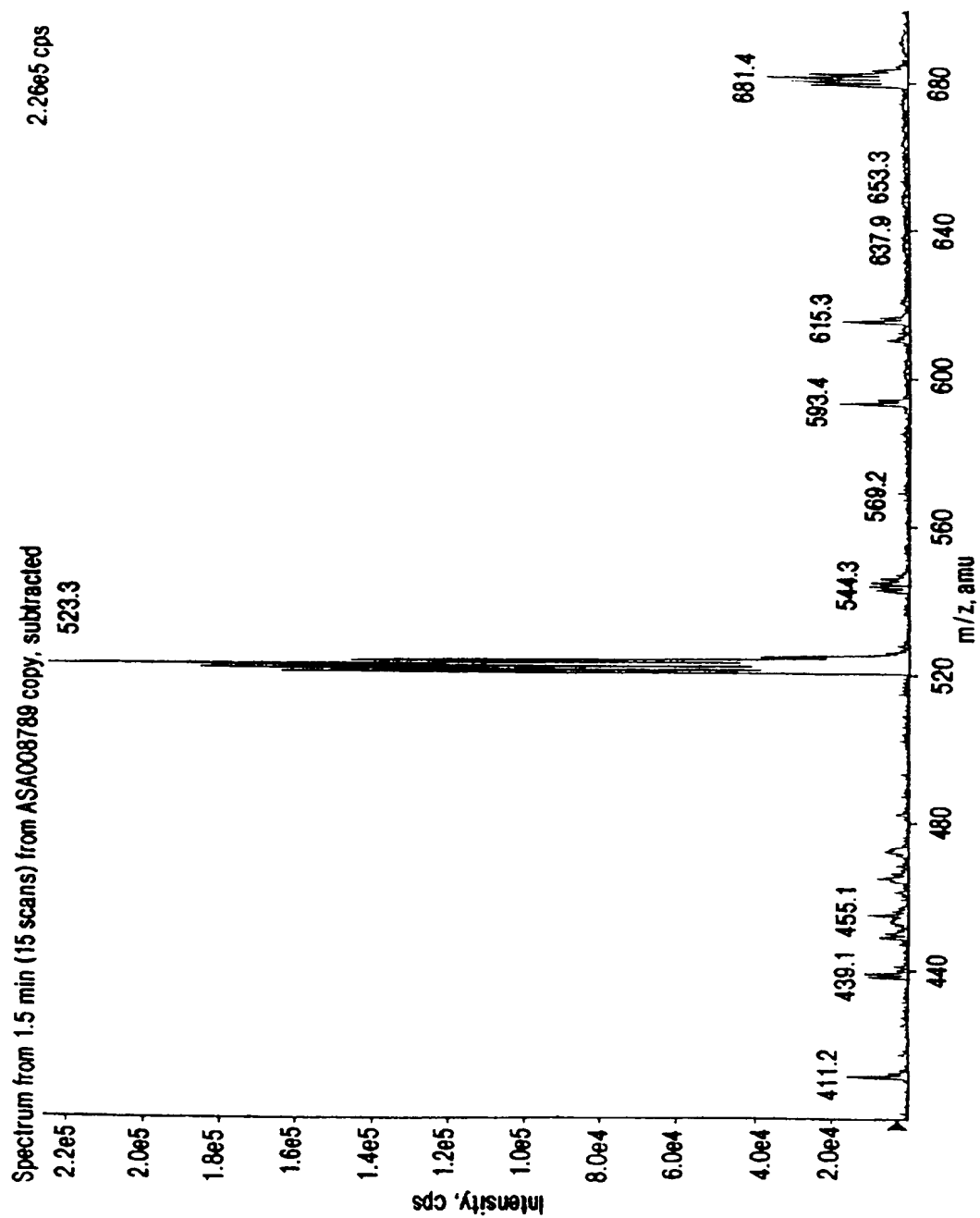


FIG. 261

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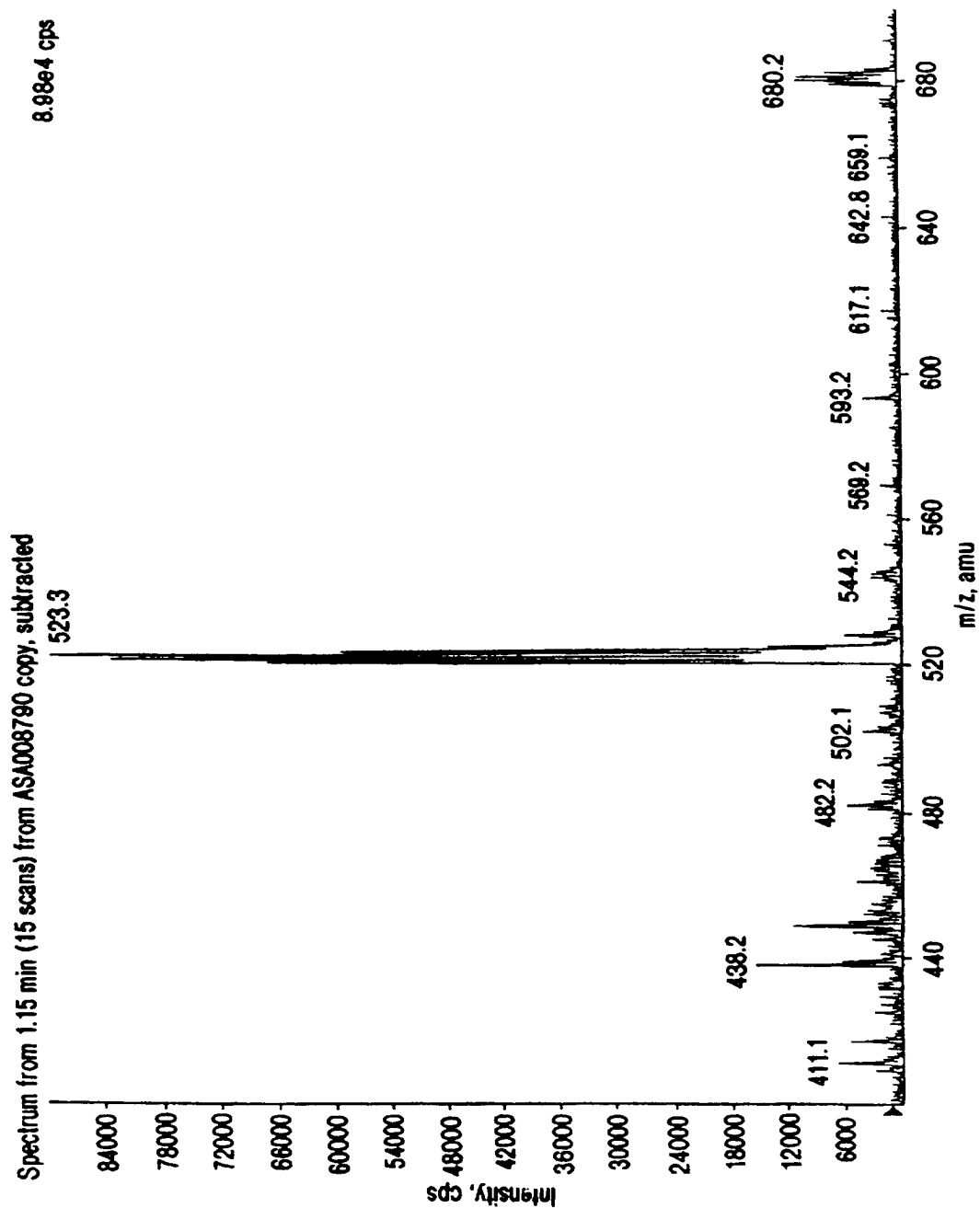


FIG. 262

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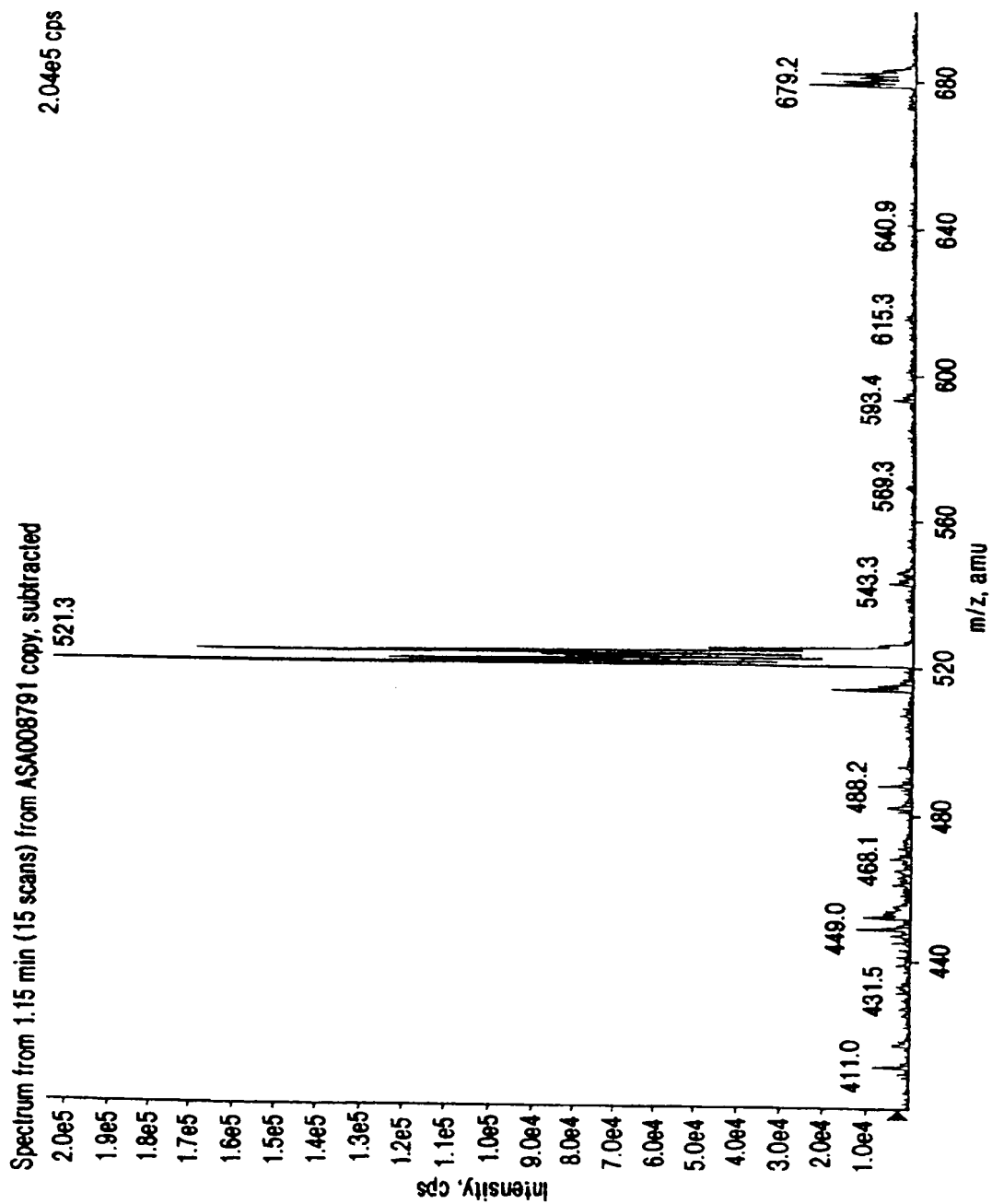


FIG. 263

SUBSTITUTE SHEET (RULE 26)

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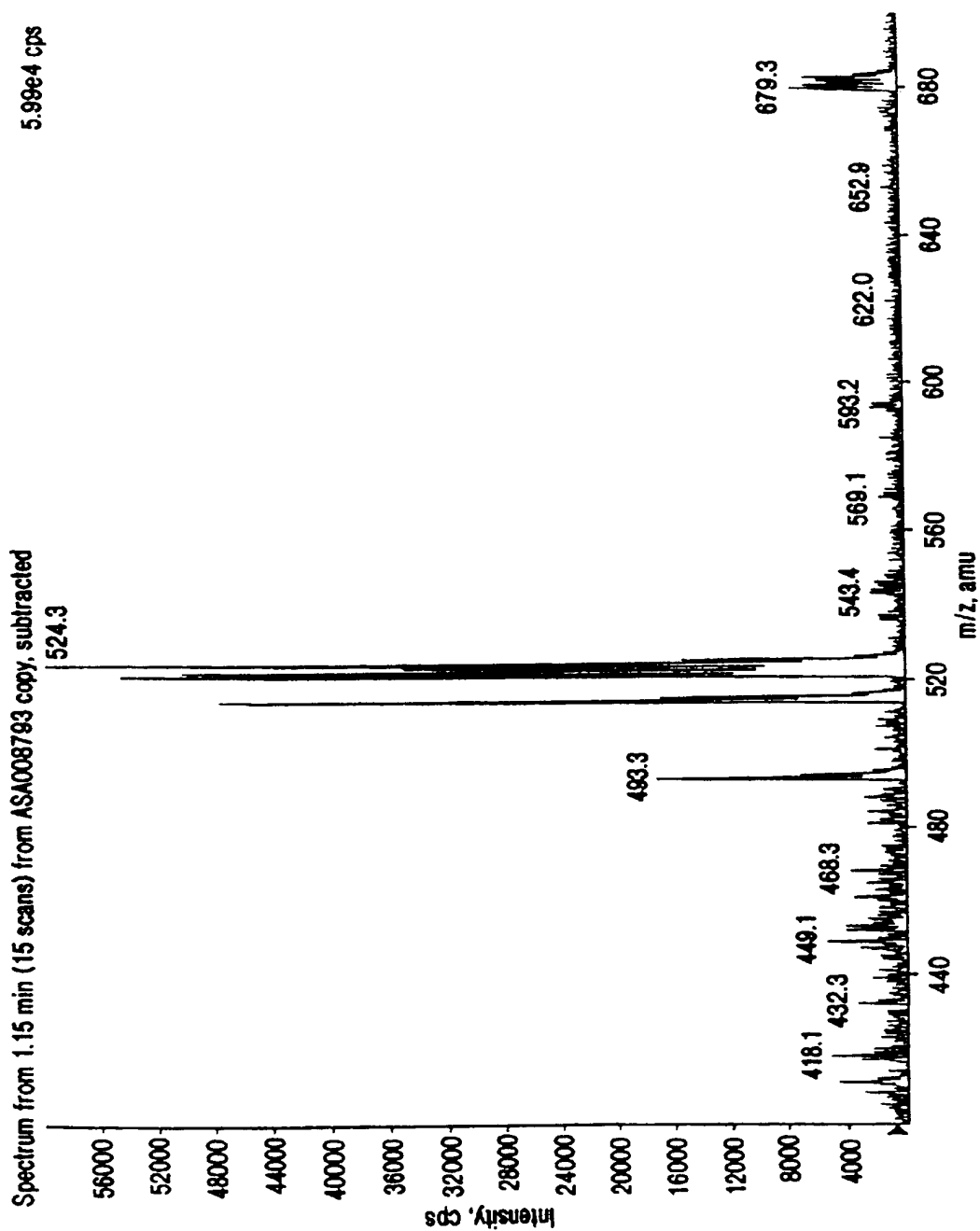


FIG. 264

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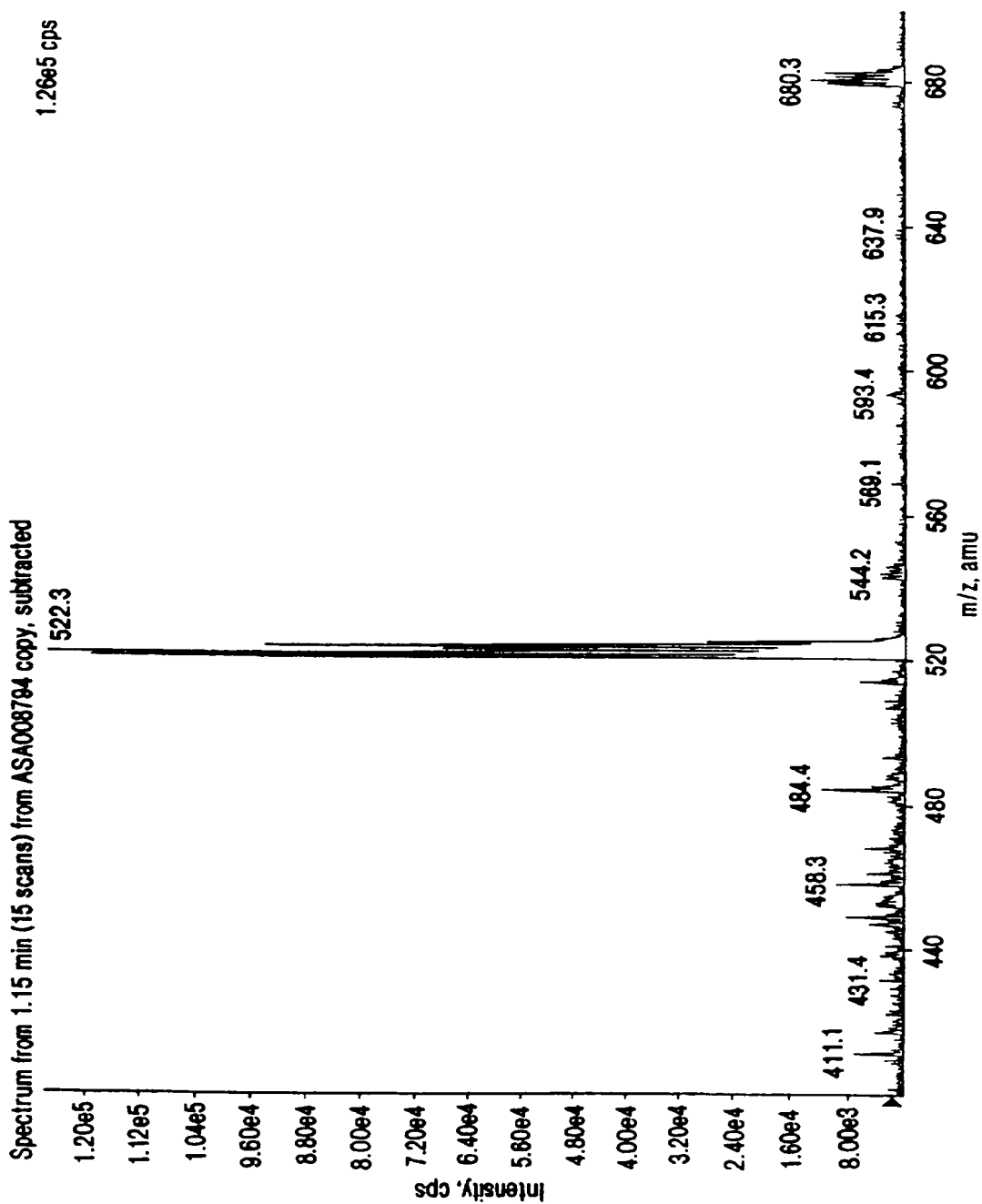


FIG. 265

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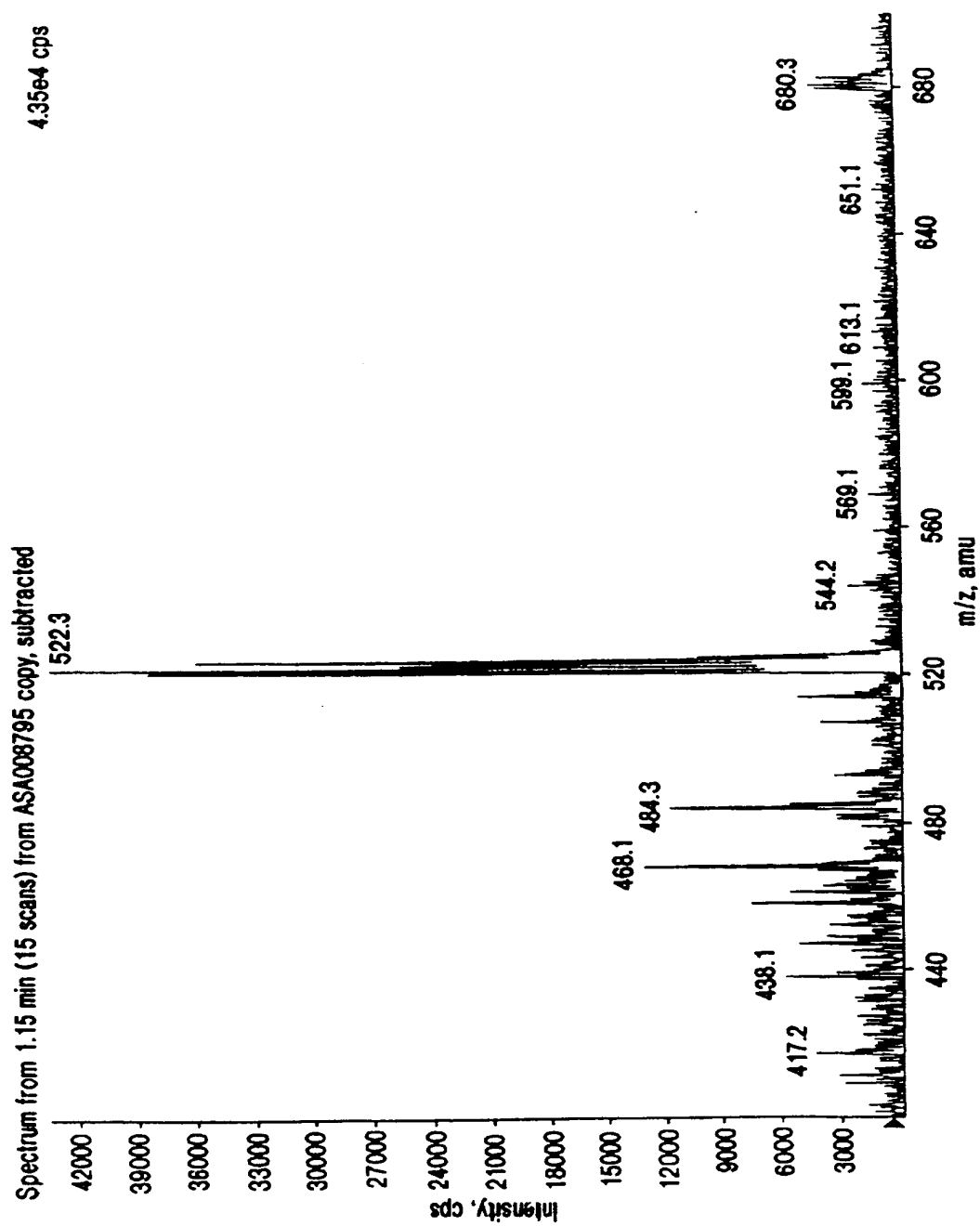


FIG. 266

SUBSTITUTE SHEET (RULE 26)

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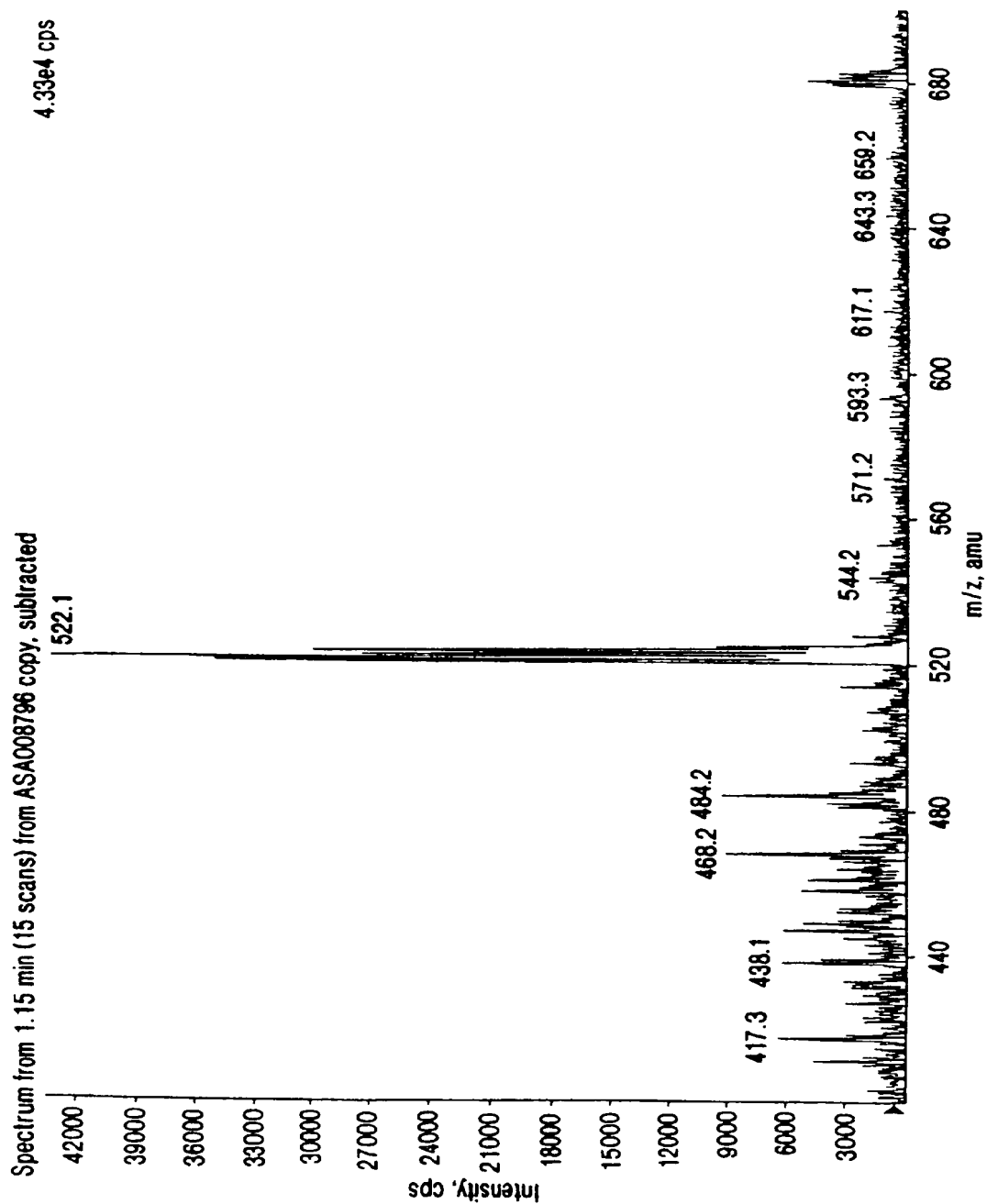


FIG. 267

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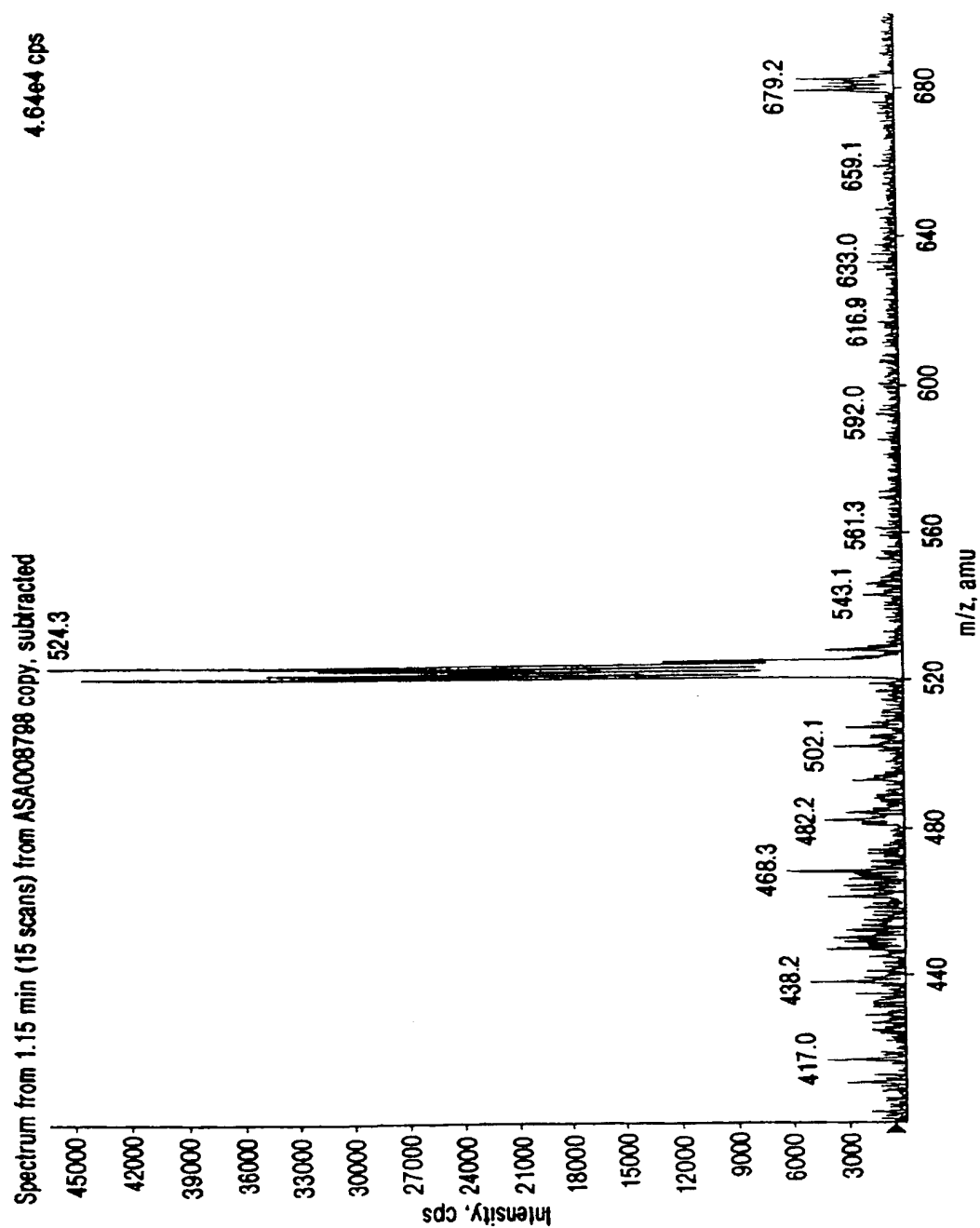


FIG. 268

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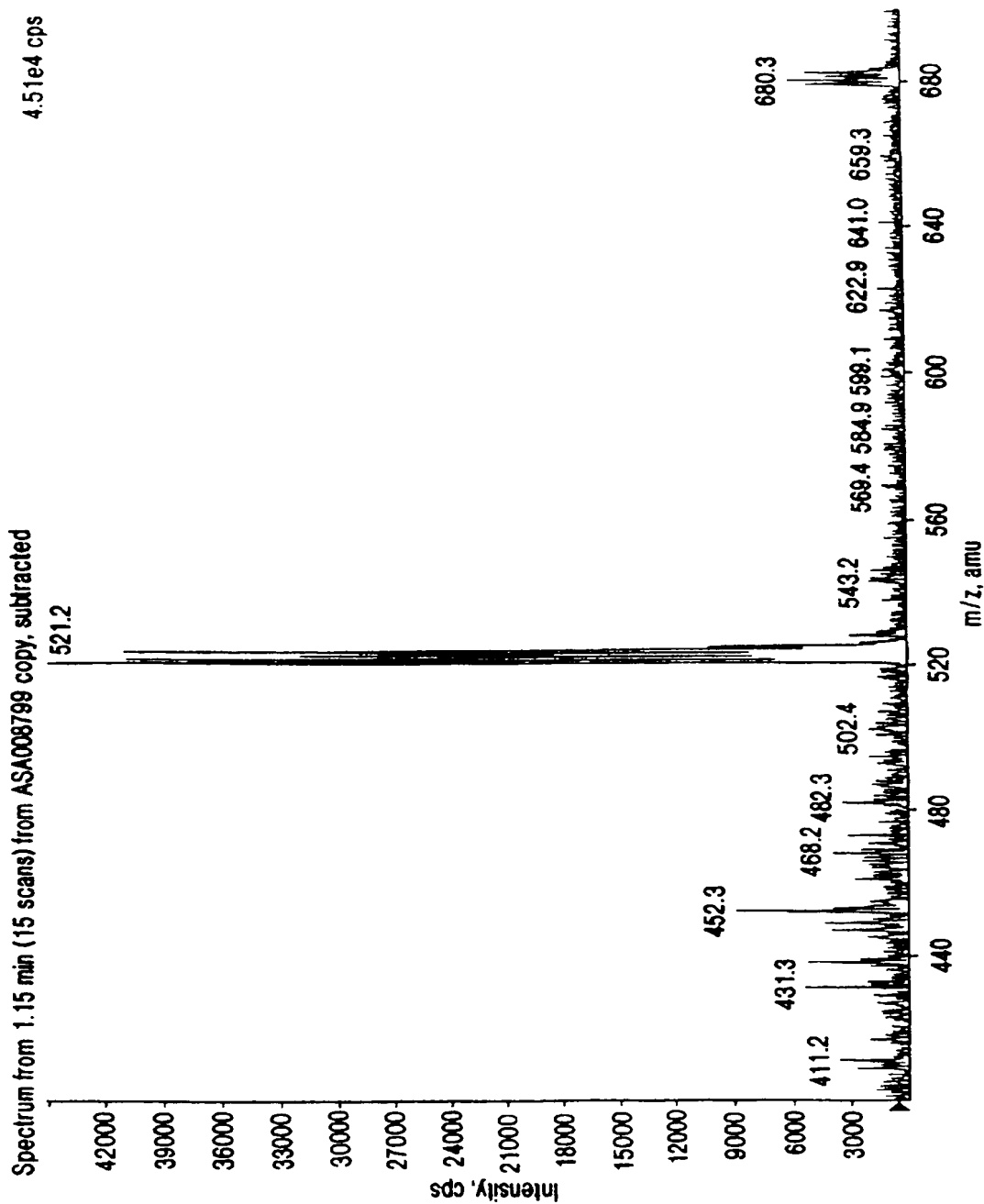


FIG. 269

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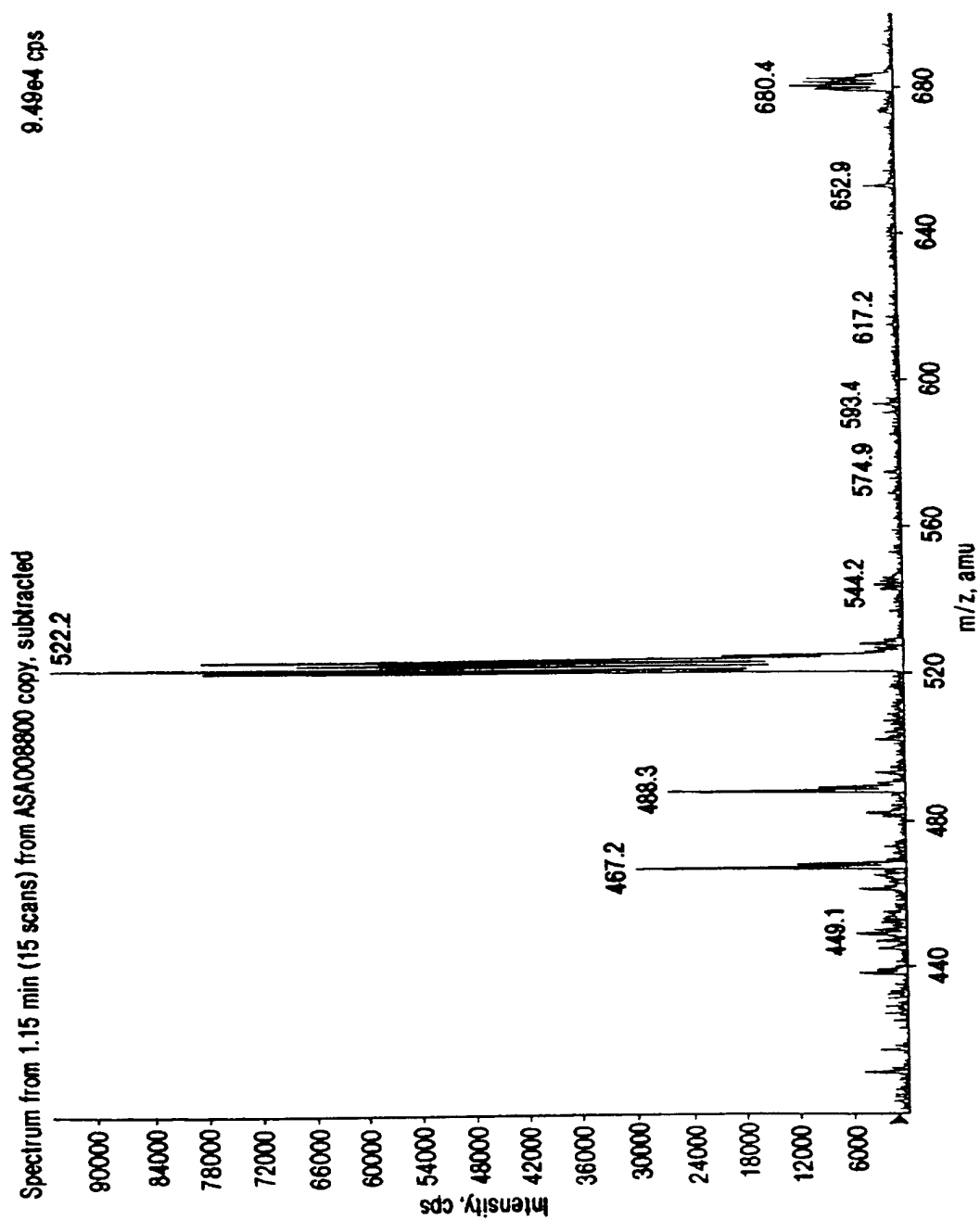


FIG. 270

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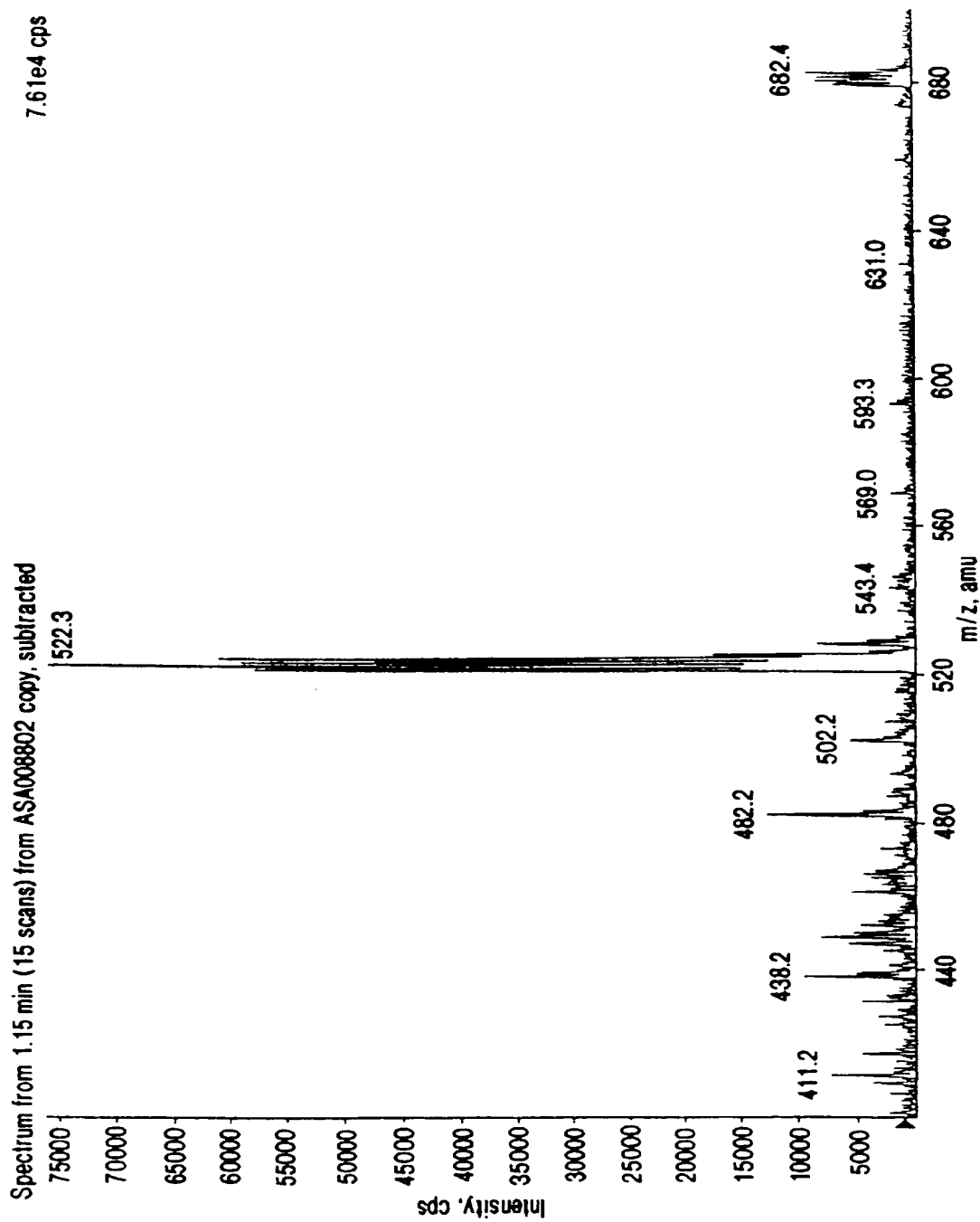


FIG. 271

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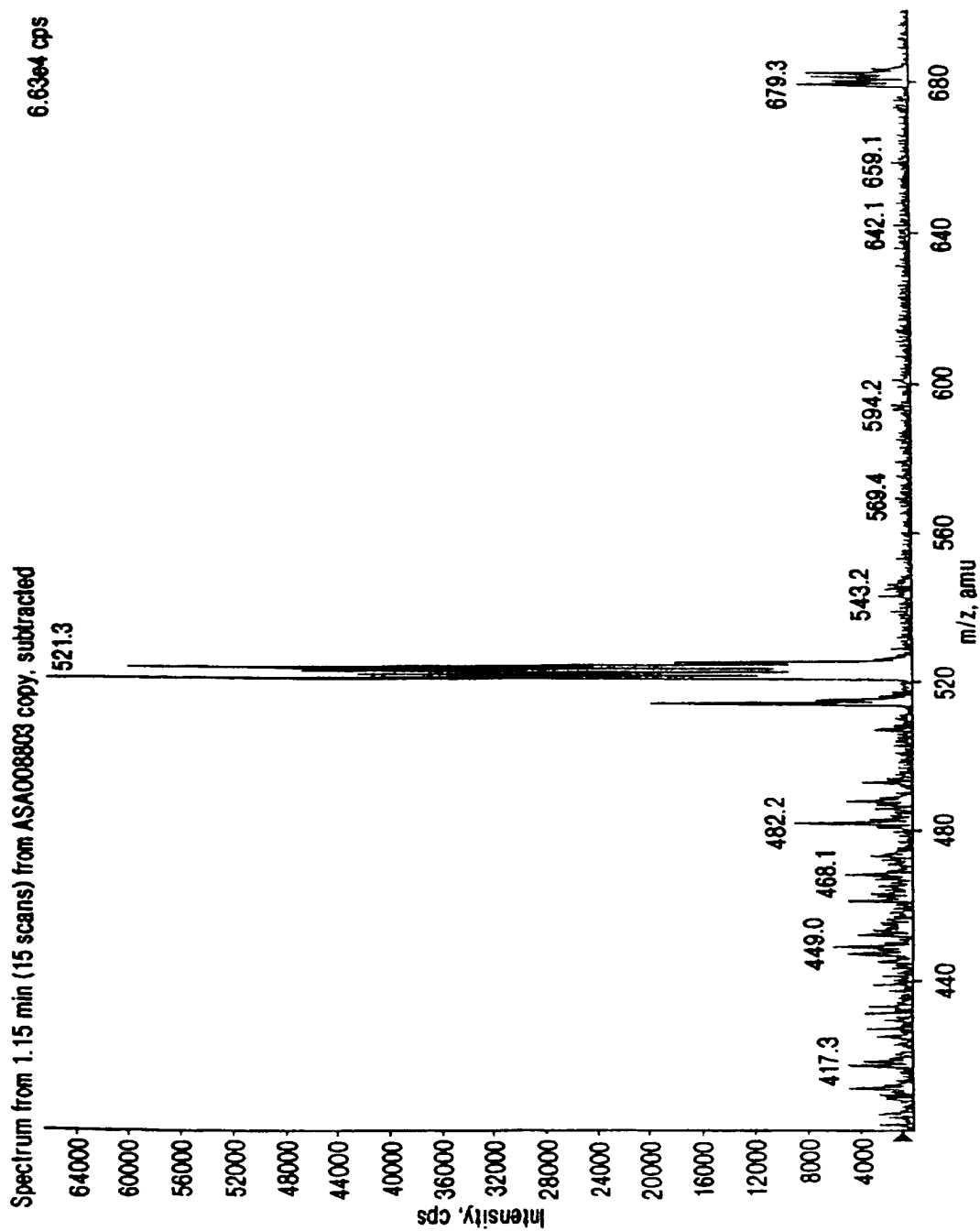


FIG. 272

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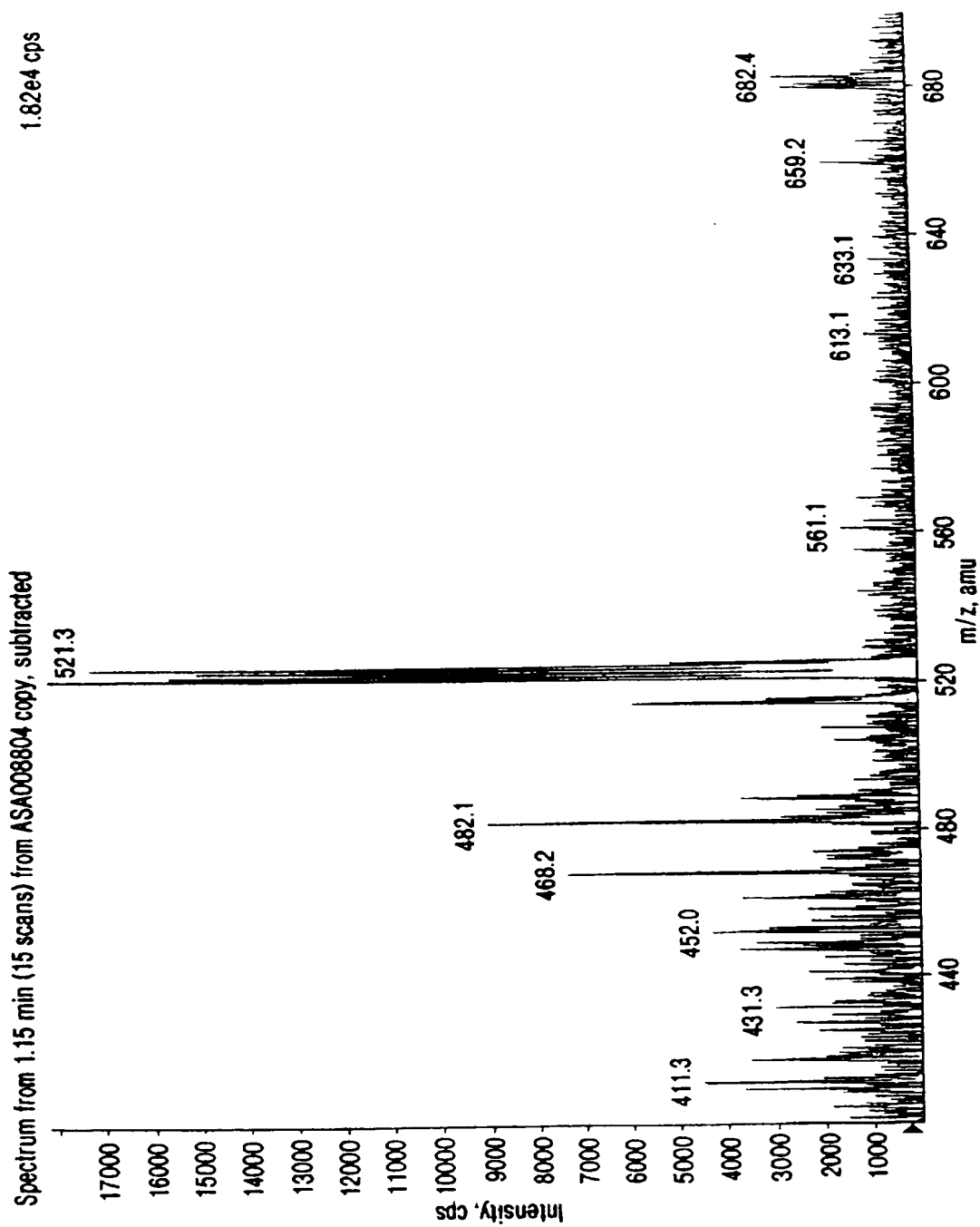


FIG. 273

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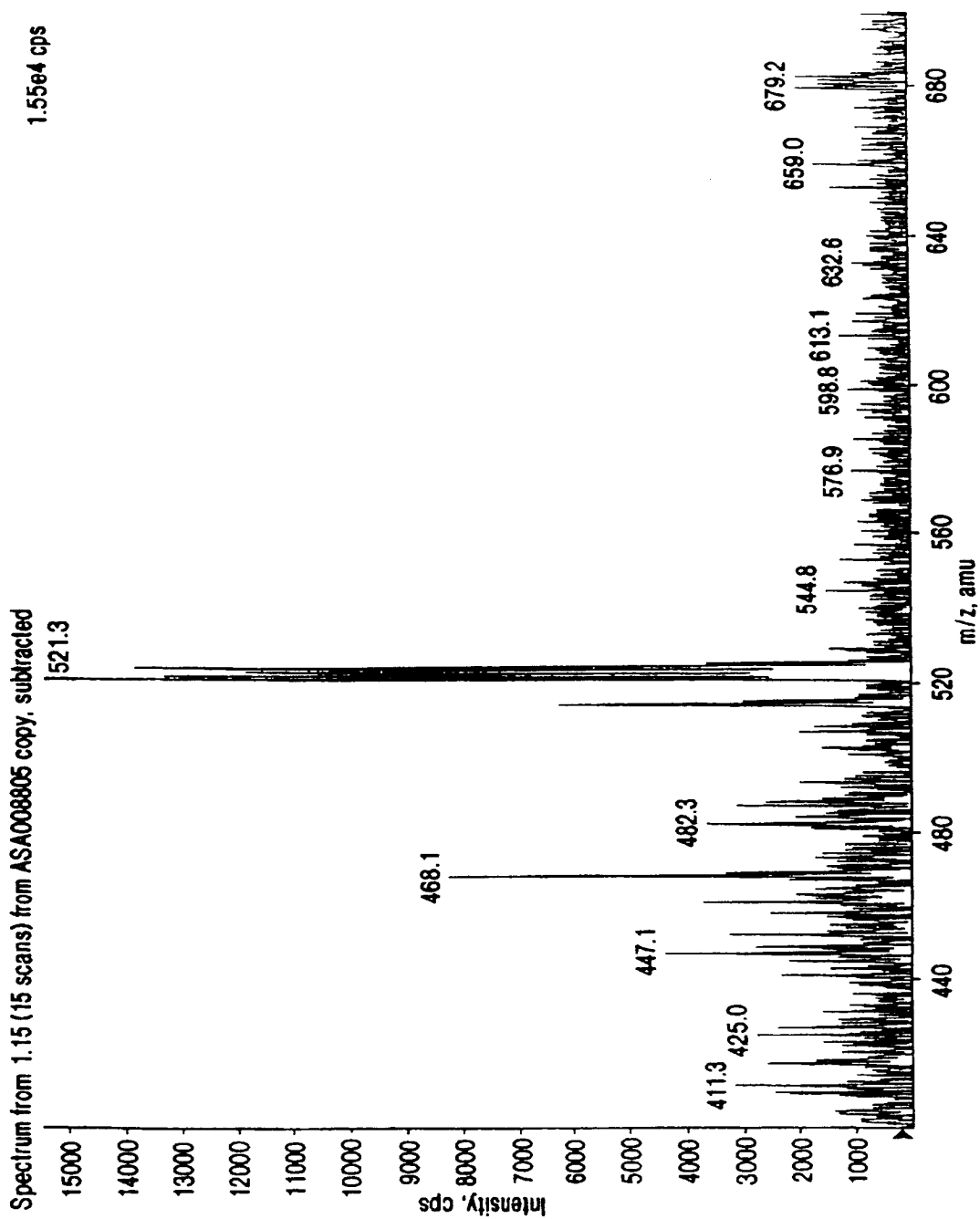


FIG. 274

SUBSTITUTE SHEET (RULE 26)

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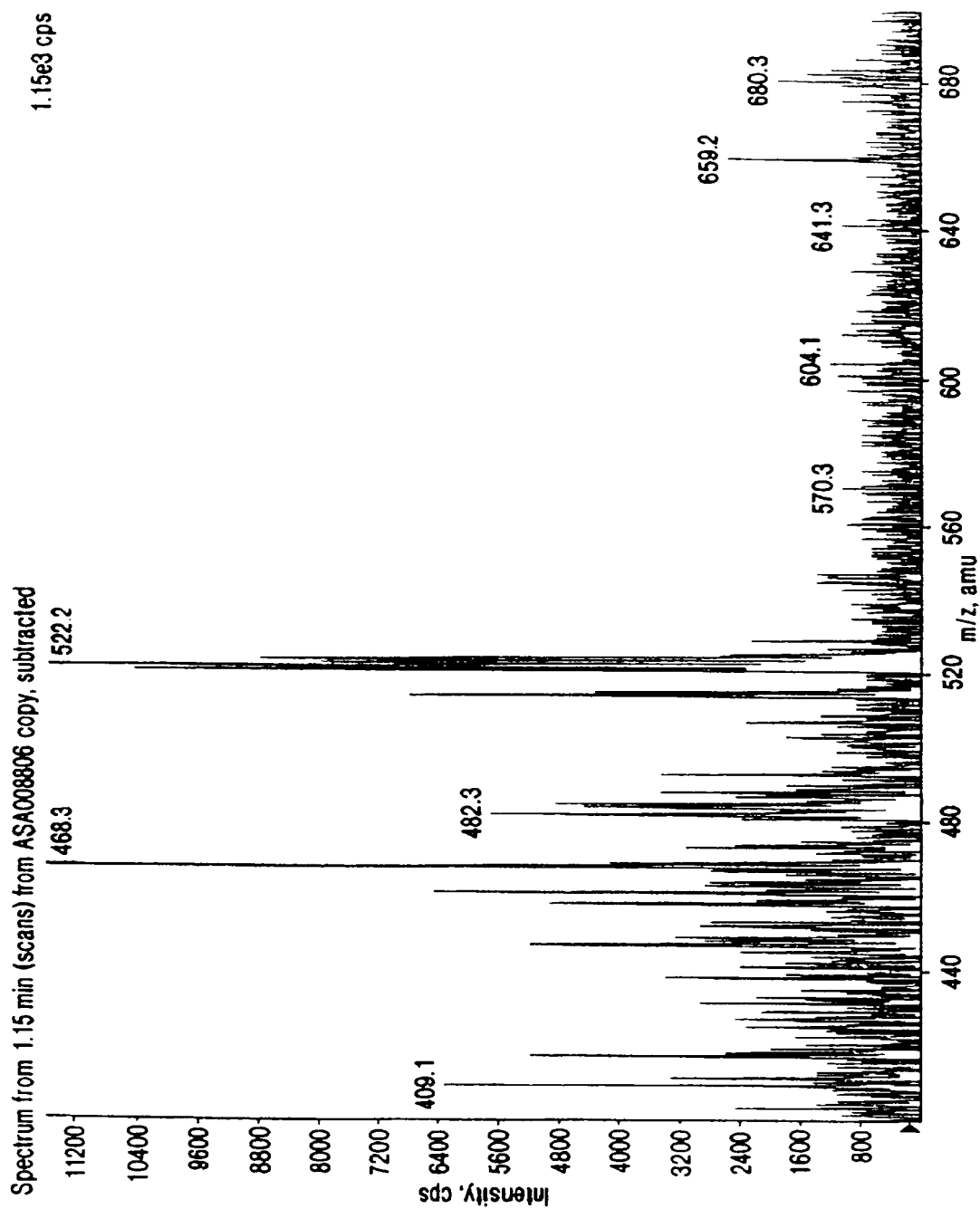


FIG. 275

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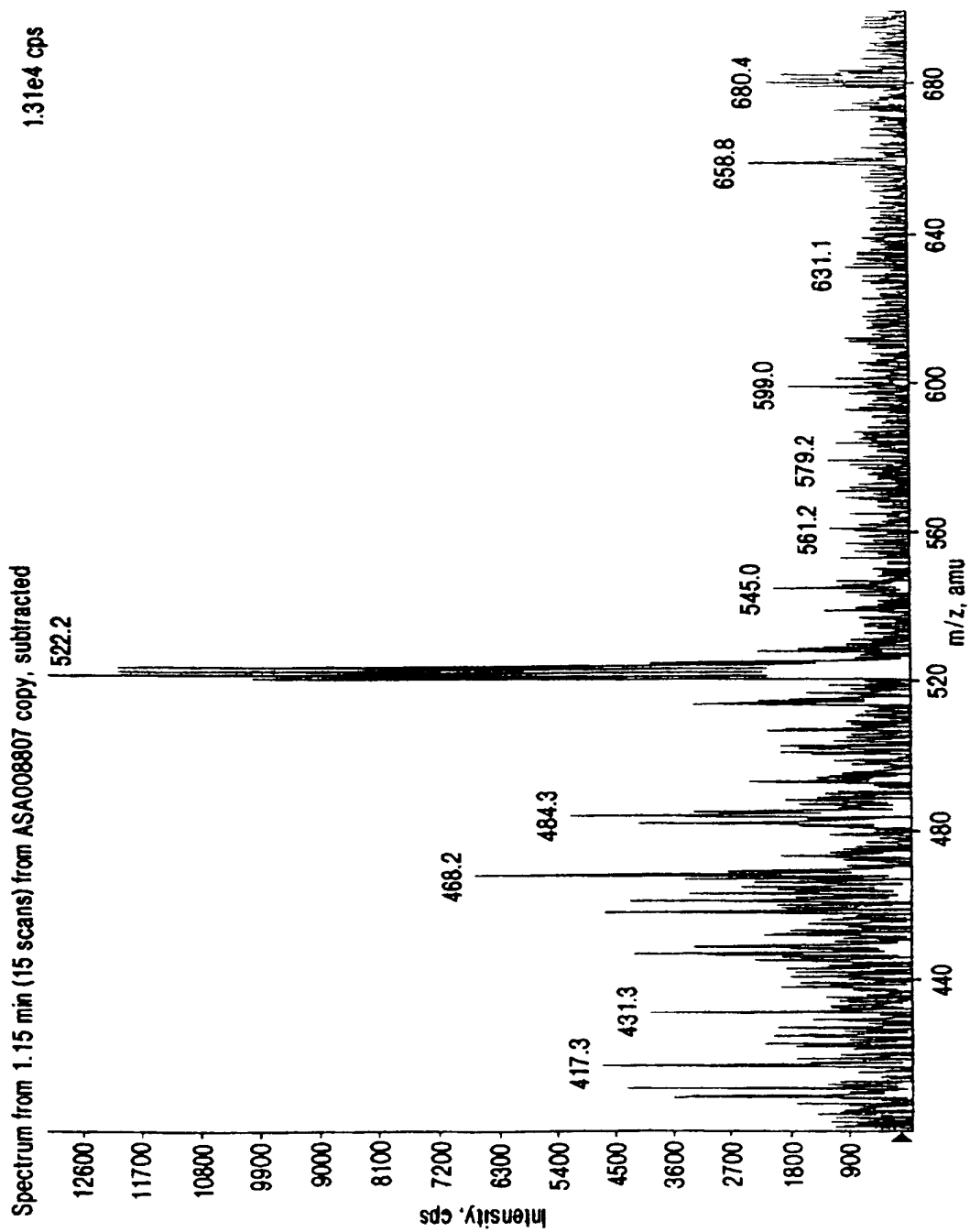


FIG. 276

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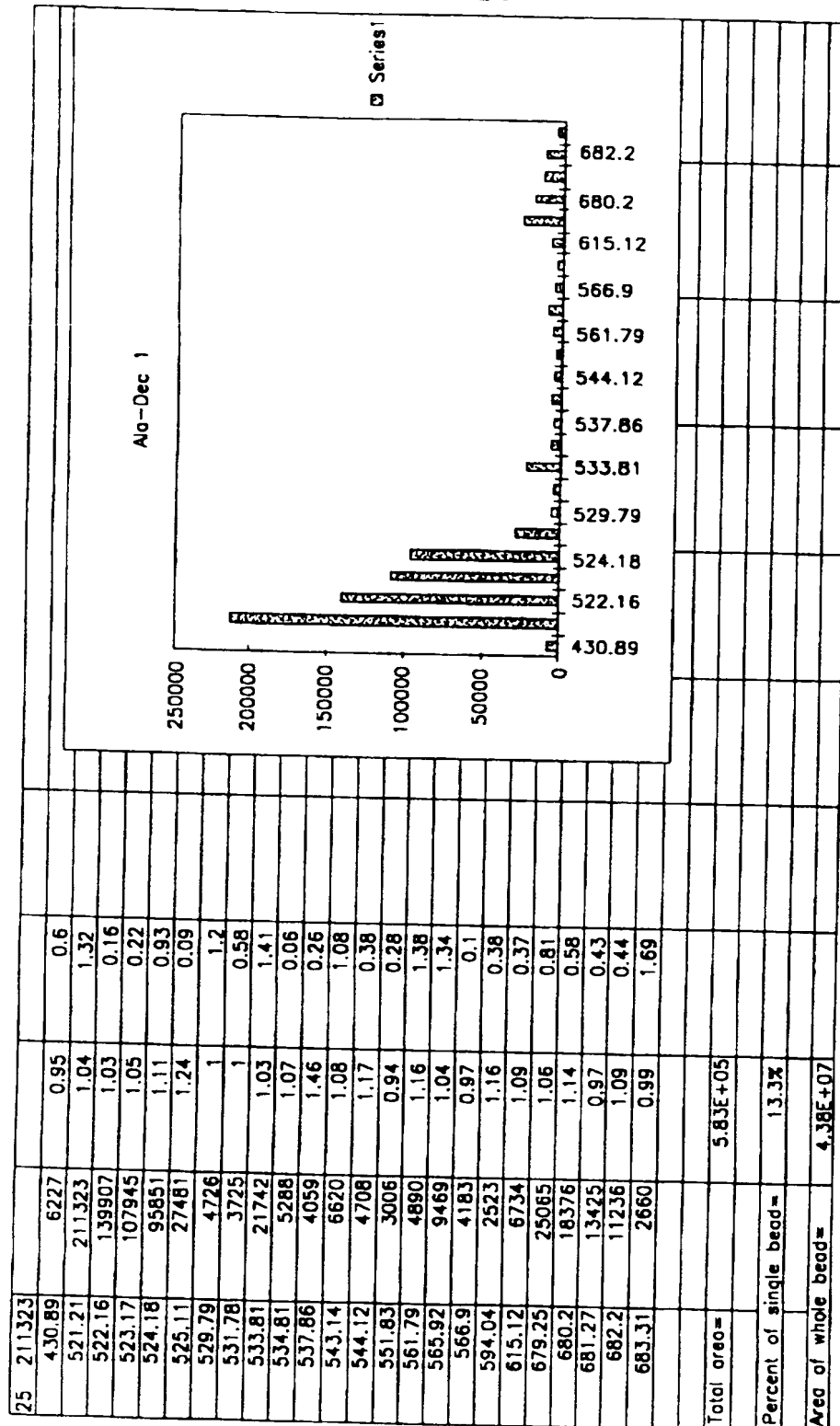


FIG. 277

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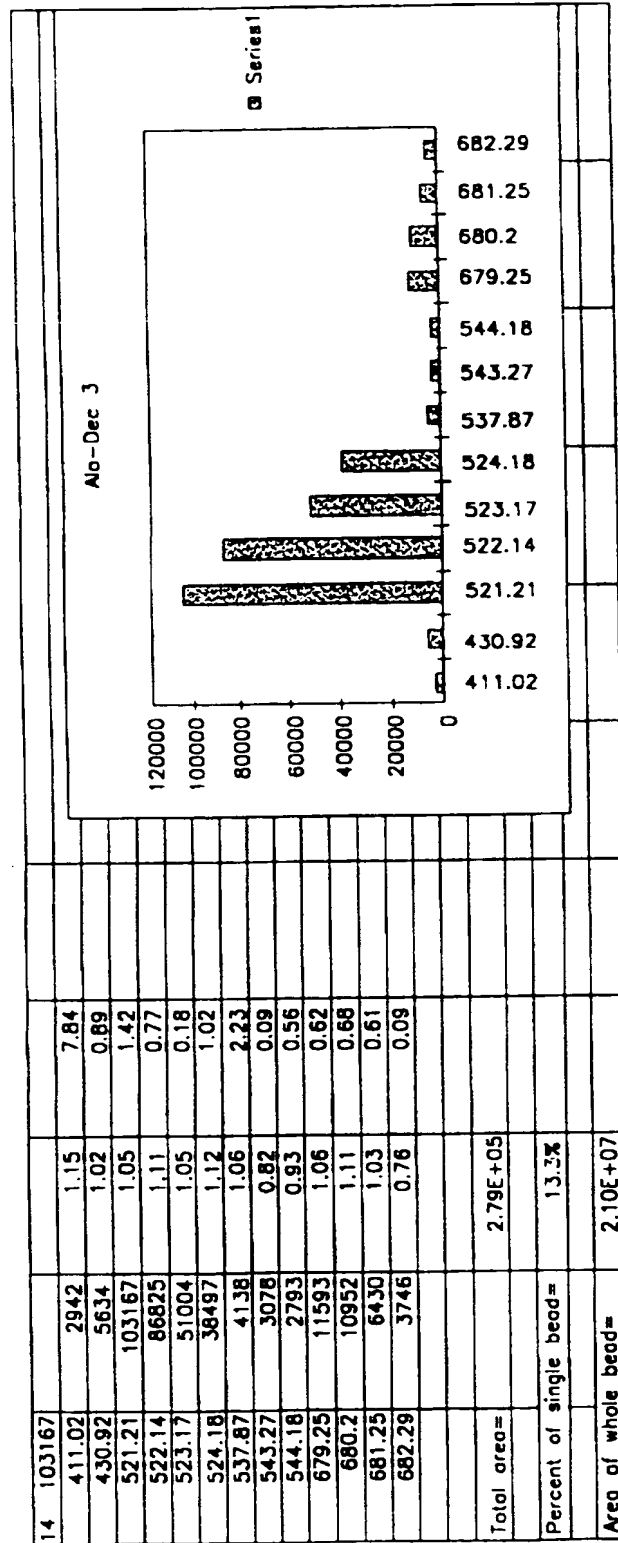
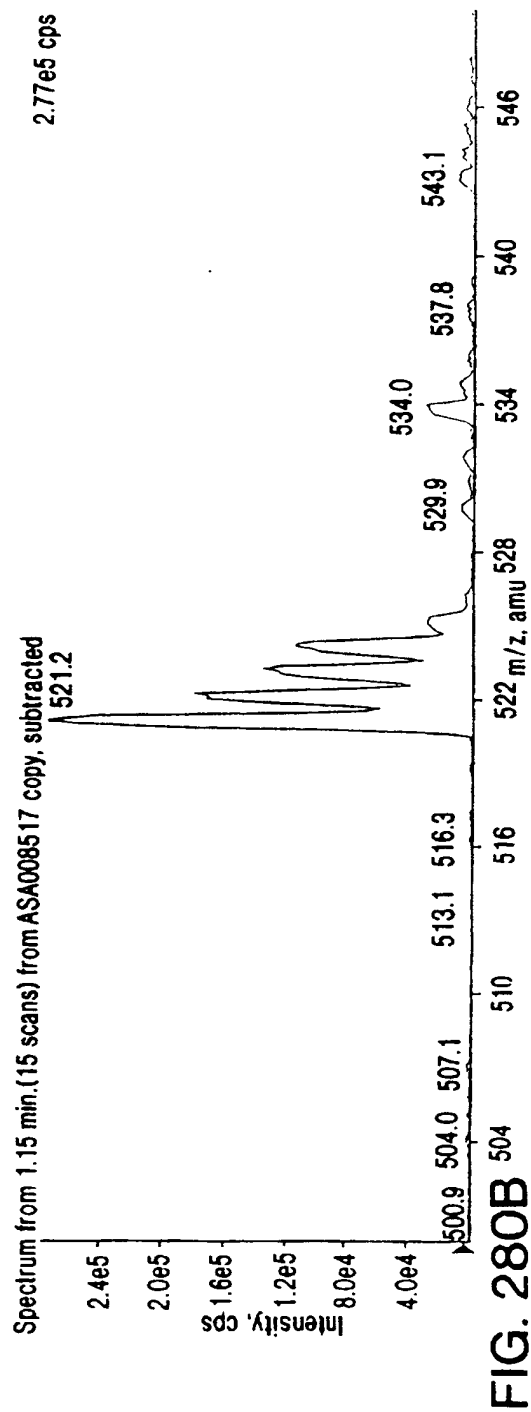
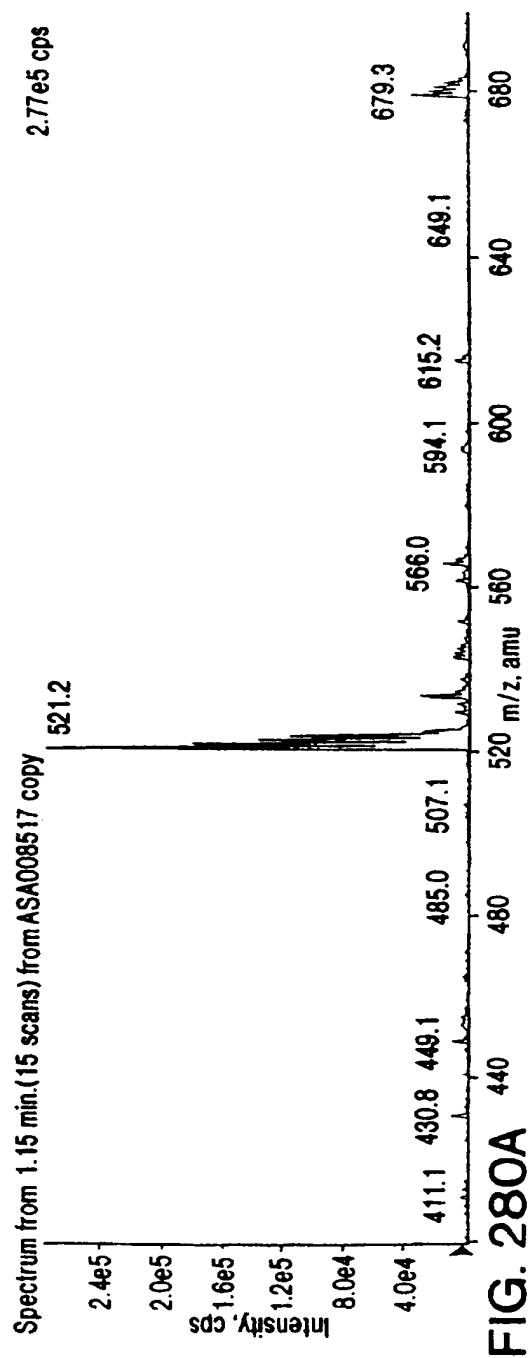


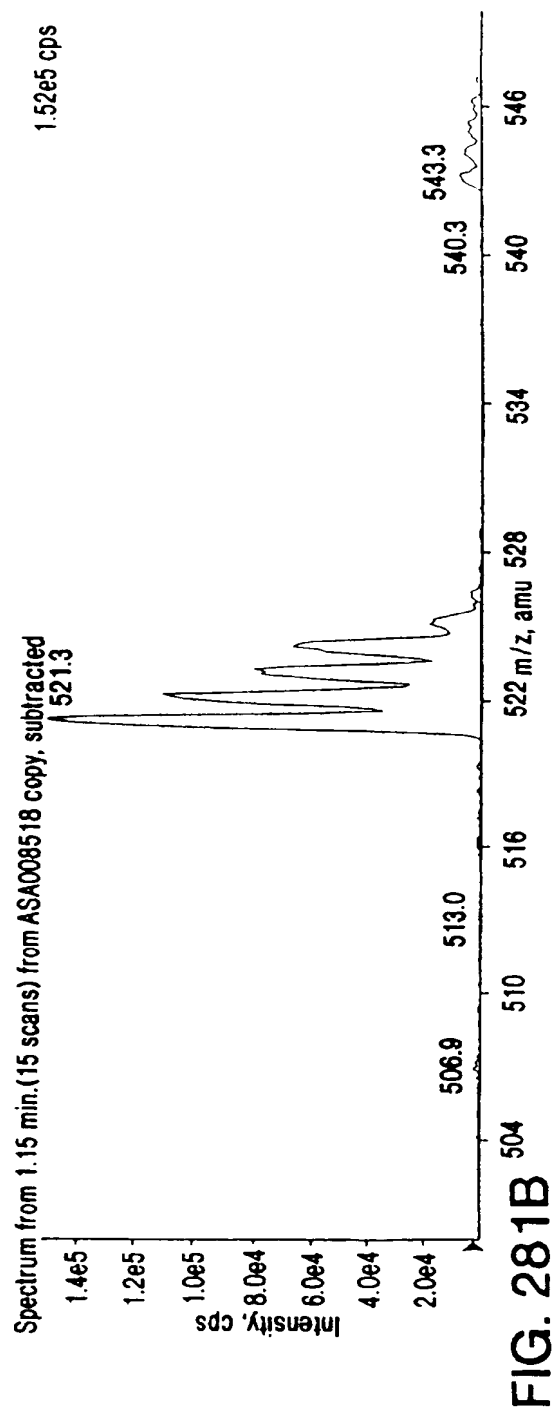
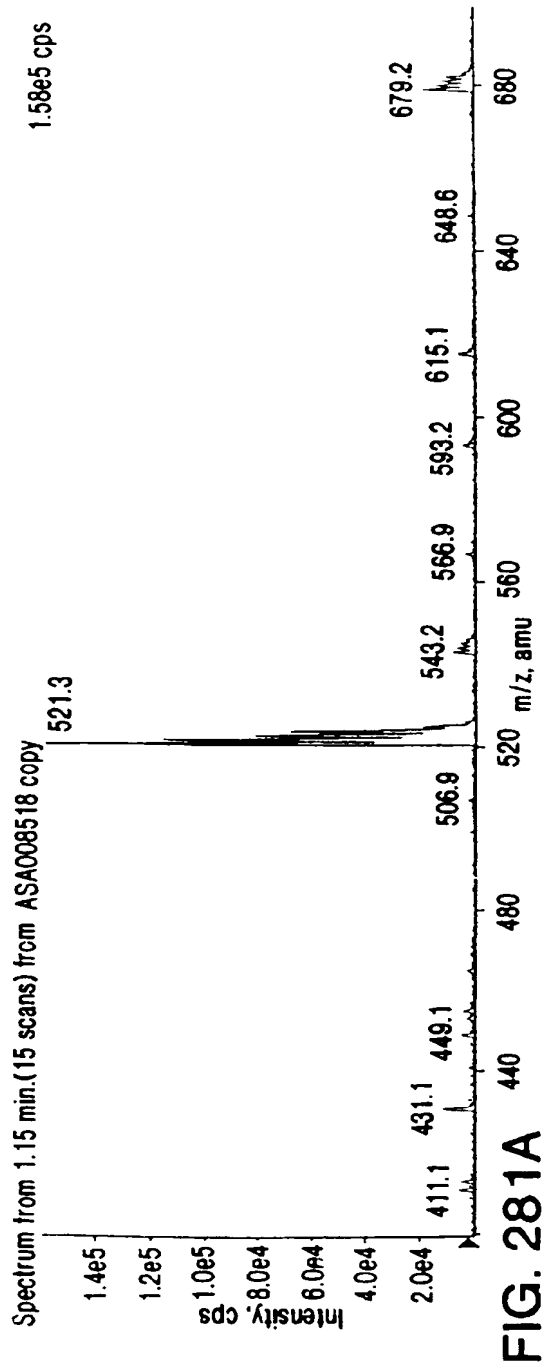
FIG. 279

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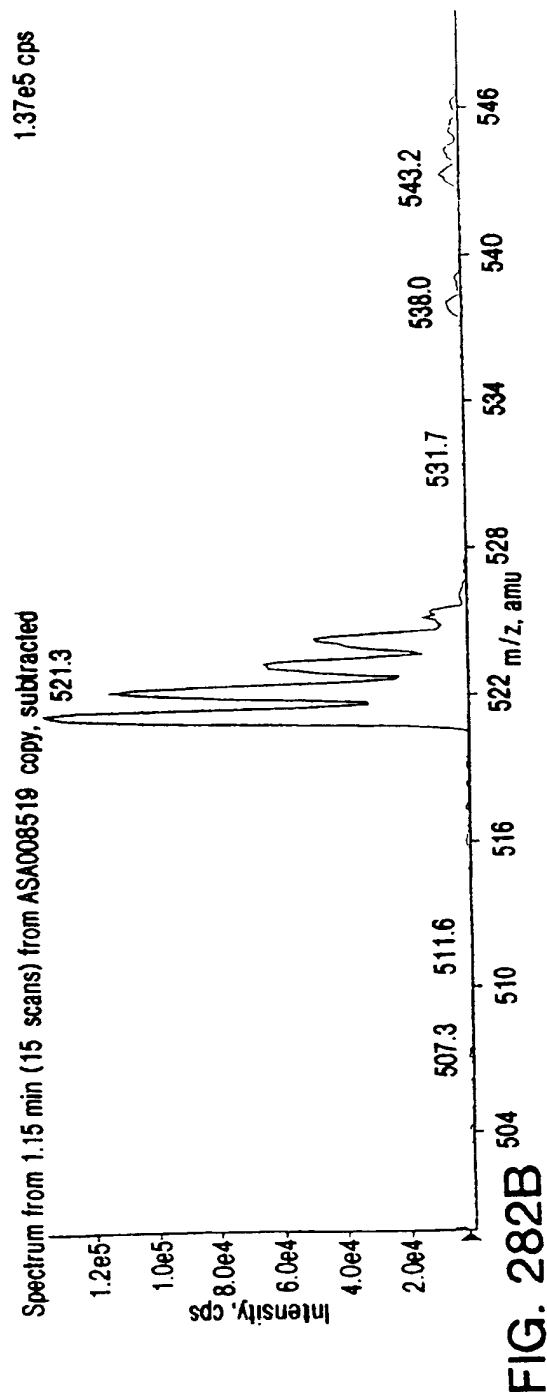
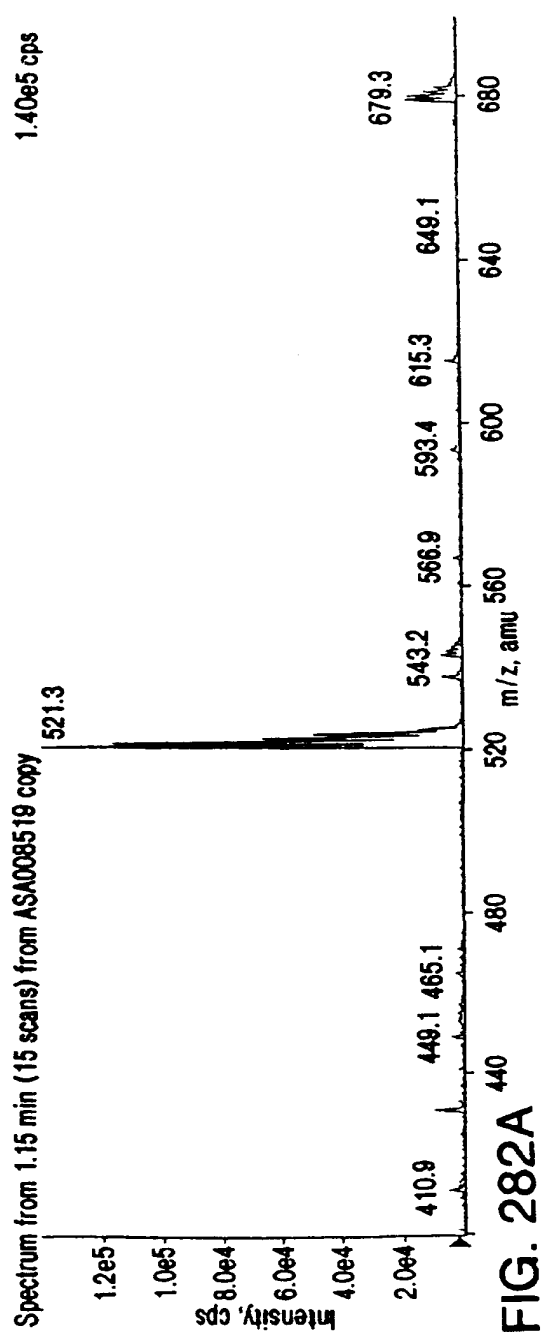


SUBSTITUTE SHEET (RULE 26)

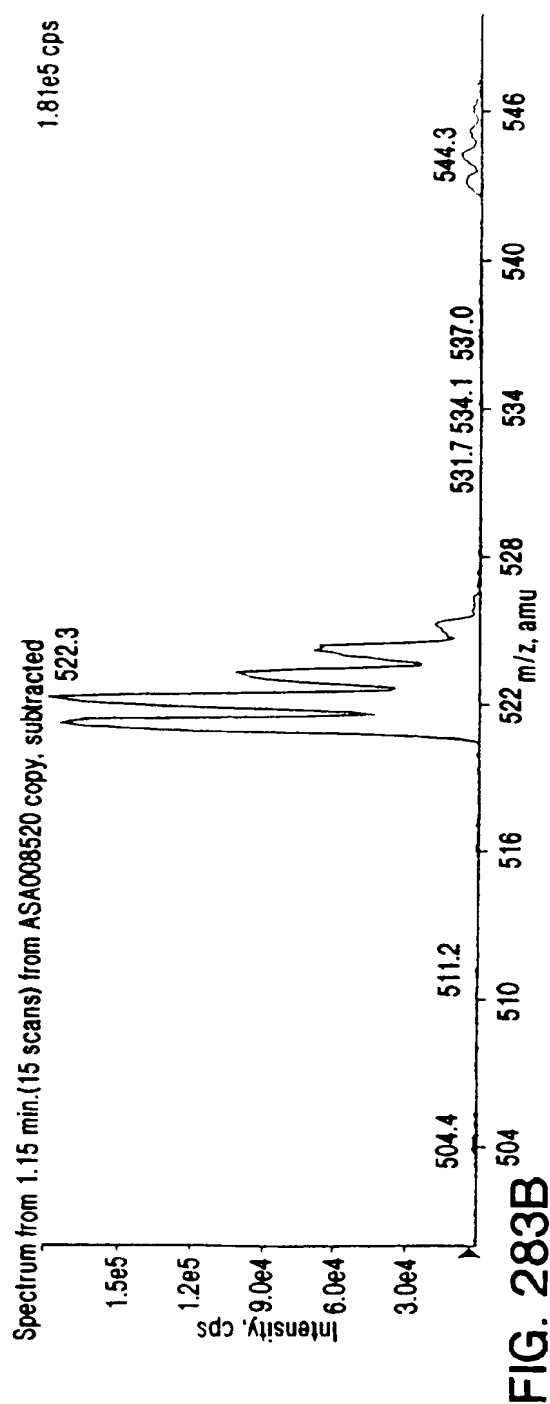
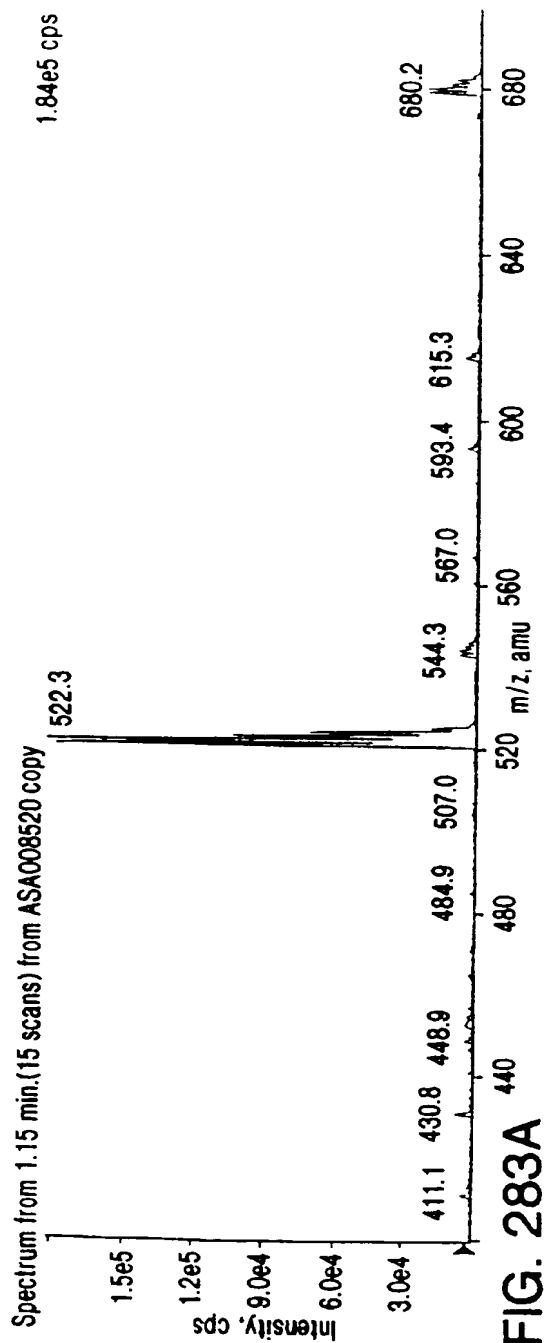
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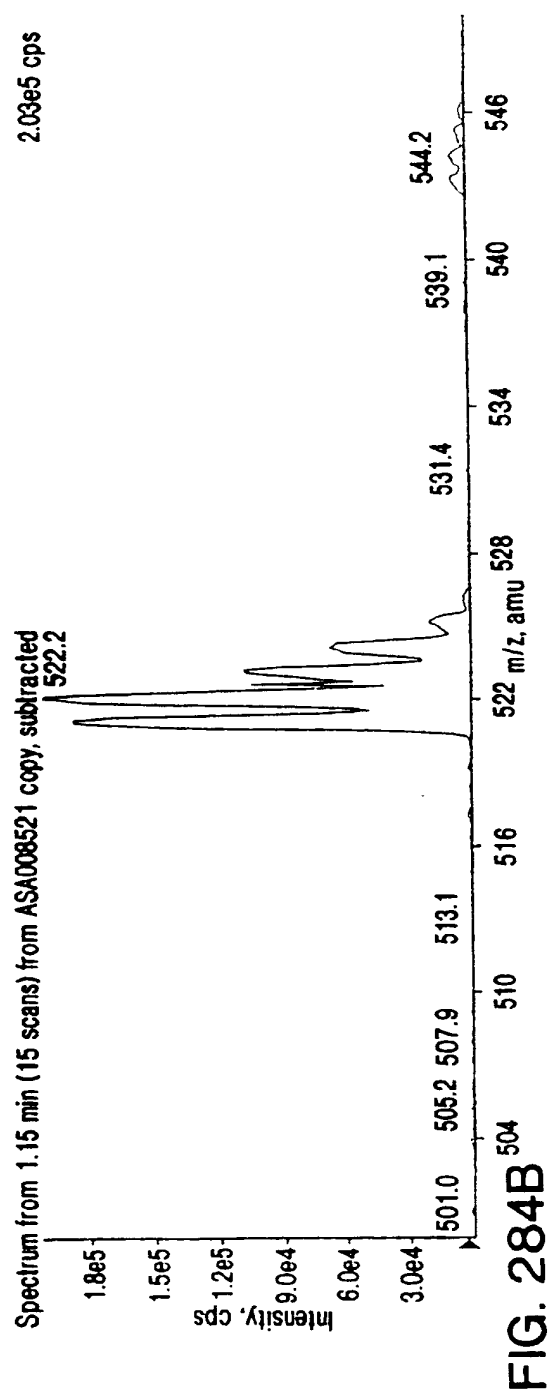
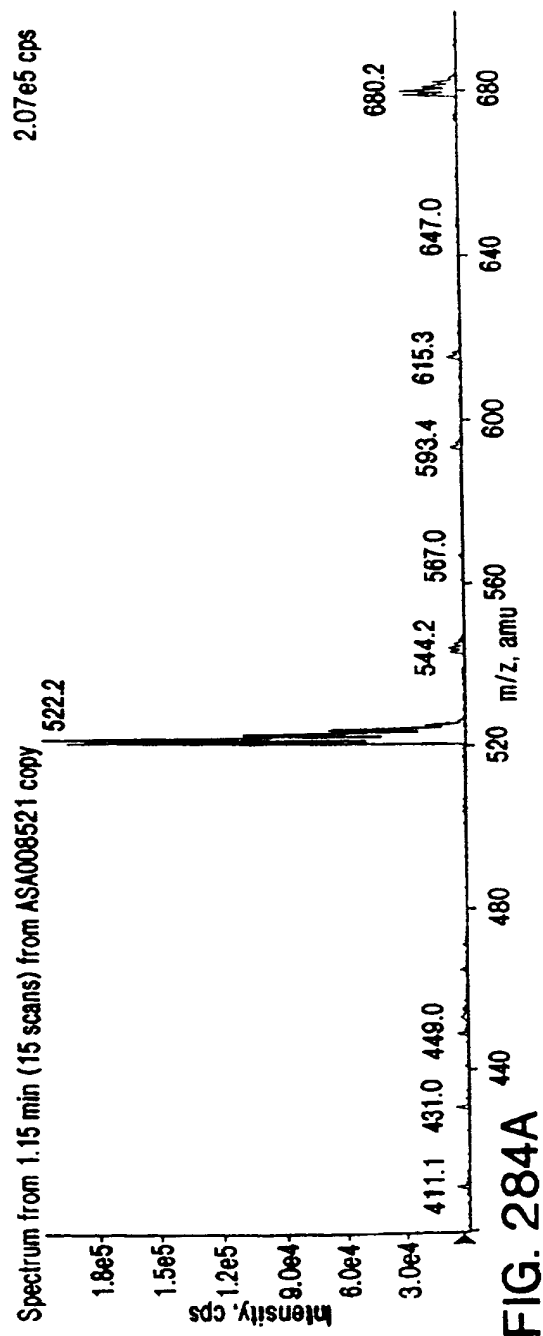
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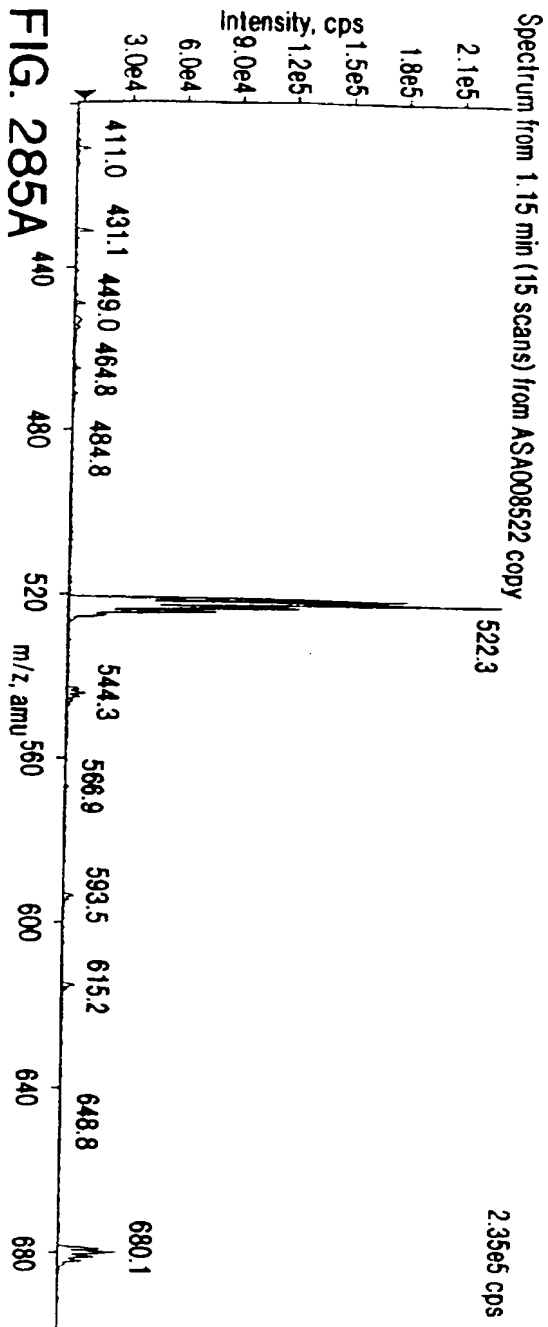


FIG. 285A

Spectrum from 1.15 min (15 scans) from ASAD08522 copy, subtracted

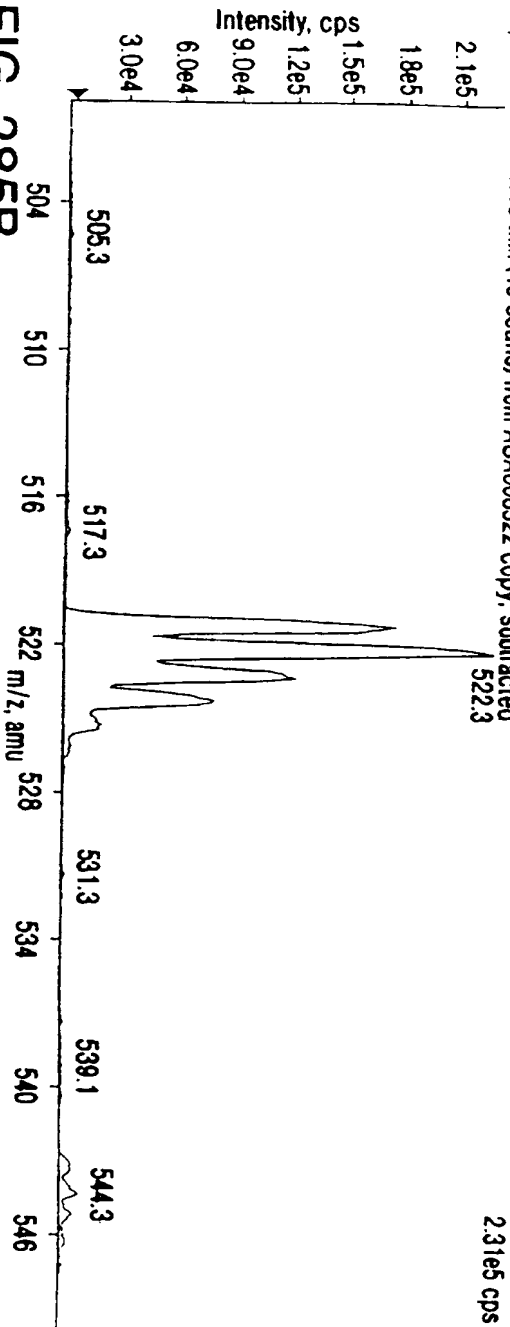


FIG. 285B

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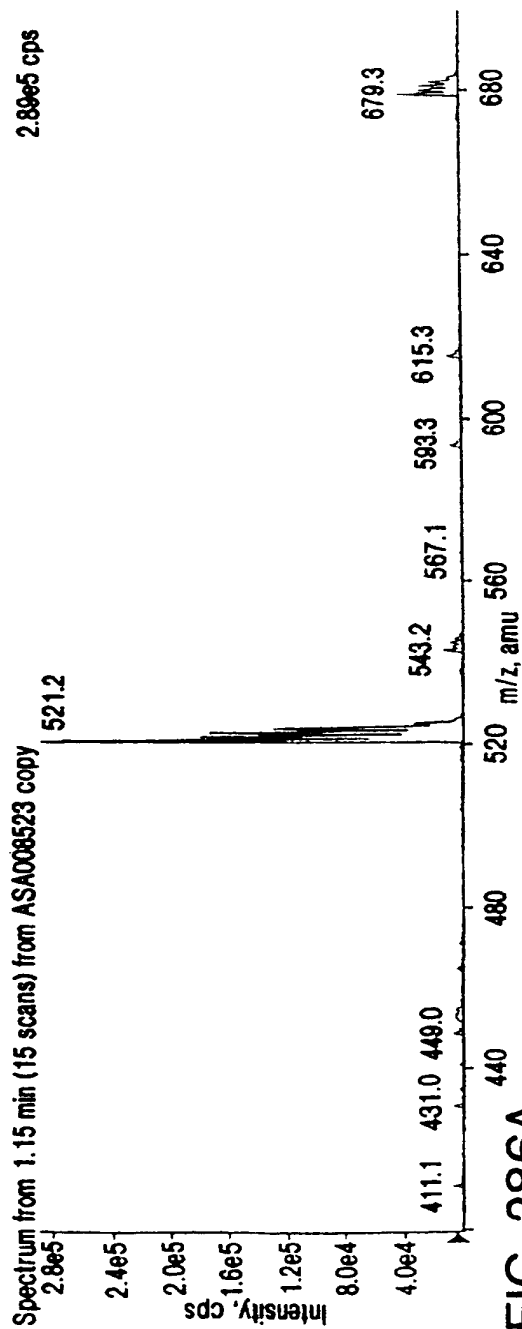


FIG. 286A

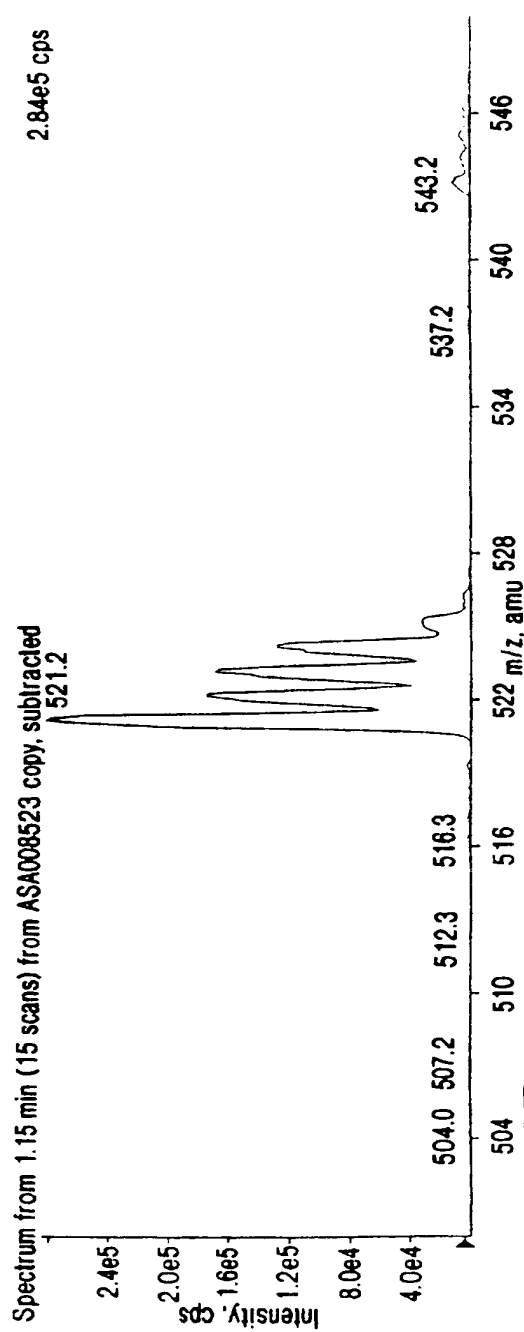


FIG. 286B

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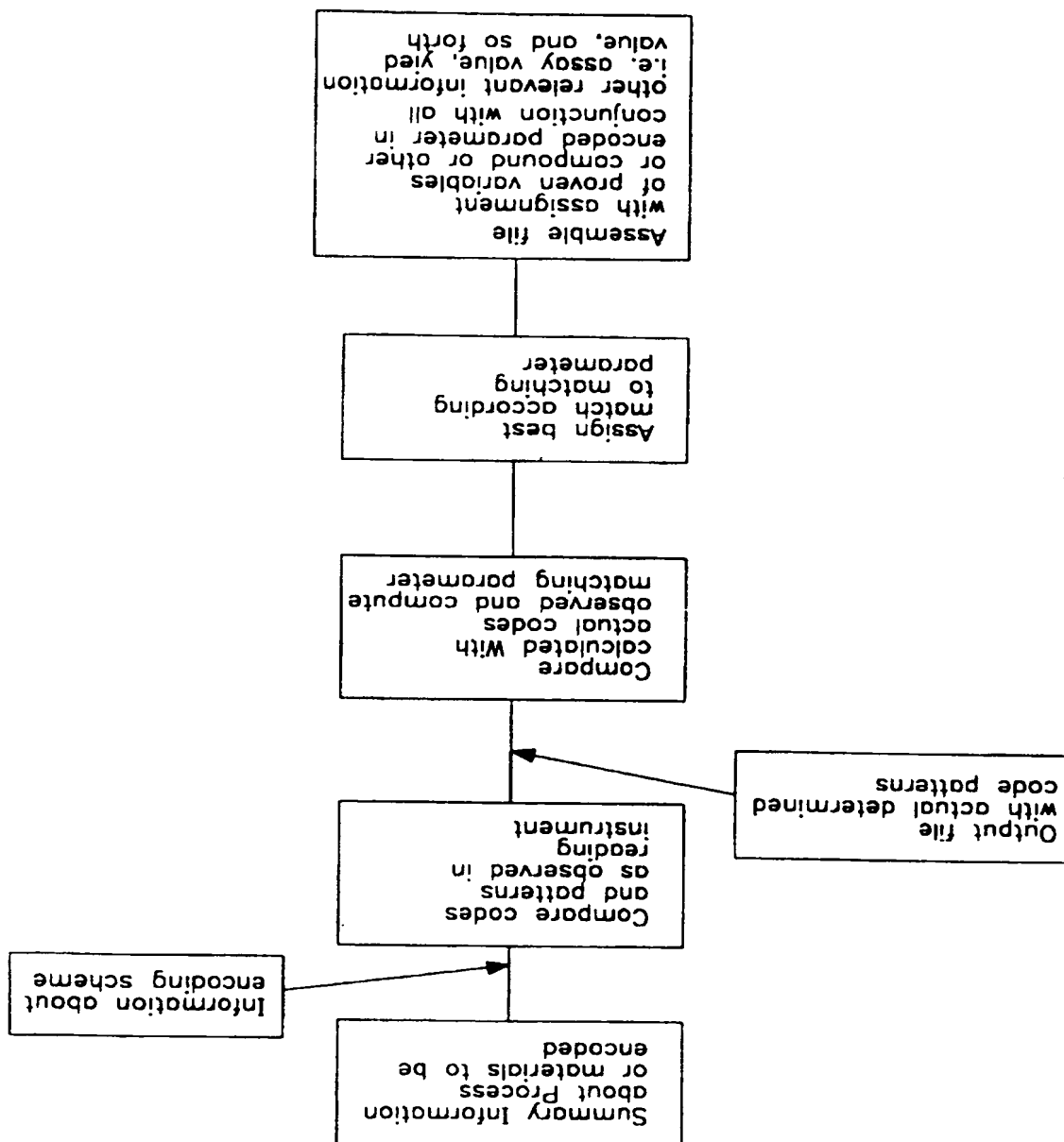


FIG. 287

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/05701

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07B61/00 C07K1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07B C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 28640 A (TRUSTEES OF COLUMBIA UNIVERSITY) 26 October 1995 see claims 1-11; page 53, line 15 to page 57, line 9; page 58, line 6 to page 59, line 29; page 64, line 31 to page 66, line 4; examples 2 and 10; pages 135-138 ---	1-11, 20-34, 47, 54-56, 62-72, 77,78
P,X	CHEMISTRY & BIOLOGY, vol. 3, no. 8, August 1996, pages 679-688, XP002035873 H. M. GEYSEN ET AL: "Isotope or mass encoding of combinatorial libraries" see the whole document --- -/-	1-78

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

23 July 1997

Date of mailing of the international search report

05.08.97

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 97/05701

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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E	WO 97 14814 A (SMITHKLINE BEECHAM) 24 Apr 11 1997 see page 2, line 15 - page 9, line 17; claims	1, 2, 12, 13
P, A	GB 2 304 410 A (ZENECA) 19 March 1997 see page 2, line 27 - page 5, line 16; claims	1, 2, 16, 17
P, X	WO 97 08190 A (SMITHKLINE BEECHAM) 6 March 1997 see page 3, line 21 - page 14, line 8; example 5	1-5, 23, 24, 31, 77, 78
P, X	WO 96 30392 A (CIBA-GEIGY) 3 October 1996 see page 2 - page 6; examples	1-4

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/05701

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9528640 A	26-10-95	US 5565324 A	15-10-96
		AU 2292695 A	10-11-95
		CA 2187792 A	26-10-95
		EP 0755514 A	29-01-97
		HU 74985 A	28-03-97
		NO 964332 A	03-12-96
WO 9714814 A	24-04-97	NONE	
GB 2304410 A	19-03-97	NONE	
WO 9708190 A	06-03-97	NONE	
WO 9630392 A	03-10-96	AU 5110096 A	16-10-96

